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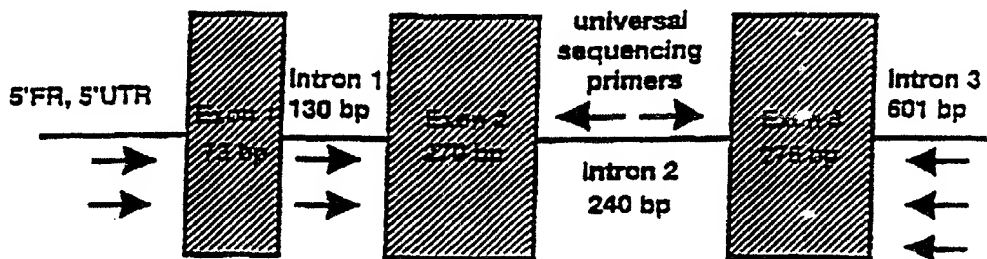
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12Q 1/68		A1	(11) International Publication Number: WO 99/07883
			(43) International Publication Date: 18 February 1999 (18.02.99)
(21) International Application Number: PCT/CA98/00768		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 11 August 1998 (11.08.98)			
(30) Priority Data: 08/909,290 11 August 1997 (11.08.97) US			
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		Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	

(54) Title: METHOD AND KIT FOR HLA CLASS I TYPING DNA

HLA class I Sequencing



group-specific non-coding region primers

(57) Abstract

The present invention relates to methods and materials for determining the HLA Class I type of a subject, wherein group-specific sequences are used to design primer molecules which may be used in amplification protocols which accurately identify the HLA group(s) and/or allele(s) carried by the subject.

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METHOD AND KIT FOR HLA CLASS I TYPING DNA

1. INTRODUCTION

The present invention relates to methods and materials for determining the HLA Class I type of a subject, wherein group-specific sequences are used to design primer molecules which may be used in amplification protocols which accurately identify the HLA group(s) and/or allele(s) carried by the subject.

2. BACKGROUND OF THE INVENTION

The Histocompatibility Locus Antigen ("HLA") Class I genes comprise three classical genes encoding the major transplantation antigens HLA-A, HLA-B, and HLA-C and seven other Class I genes of which HLA-E, HLA-F and HLA-G are probably functional genes and HLA-H, HLA-I, HLA-K and HLA-L are pseudogenes. The class I genes share a similar structure, which includes, *inter alia*, 5' -> 3', a 5' untranslated flanking region; a first exon ("exon 1") having a length of approximately 73 base pairs ("bp"); a first intron ("intron 1") having a length of approximately 130 bp; a second exon ("exon 2"), having a length of approximately 250 bp; a second intron ("intron 2"), having a length of approximately 272 bp; a third exon ("exon 3"), having a length of approximately 276 bp; a third intron ("intron 3"), having a length of approximately 588 bp; and a fourth exon ("exon 4").

The HLA Class I genes are highly polymorphic among individuals. As of 1996, at least 73 alleles of HLA-A, 126 alleles of HLA-B and 35 alleles of HLA-C have been identified. This variability is of particular relevance when tissue transplantation between a donor and a host is contemplated. The histocompatibility antigens of donor and host should be as similar as possible to avoid both immune rejection of the transplanted tissue as well as graft-versus-host disease. It is therefore important to accurately identify the HLA types of donor and host. In view of the exigencies implicit in tissue transplantation, it is desirable that the typing be accomplished as efficiently as possible.

Methods for determining alleles of HLA-A, HLA-B, and HLA-C in a patient sample have been heavily investigated because of the functional importance of these genes in transplant tissue matching and autoimmune diseases. The first tests

developed used immunological methods to identify epitopes expressed by various HLA loci. These tests (*e.g.*, the complement-dependent cytotoxicity assay described in Terasaki and McClelland, *Nature*, 204:998, (1964)) identified broad serological specificities but were not capable of distinguishing between allelic members of a group, and sometimes mis-identified groups altogether. Unfortunately, even the most accurate of such low resolution assays cannot detect and distinguish all functionally significant transplant antigens (Anasetti et al. *Hum. Immunol.*, 29:70 (1990)).

High resolution tests performed at the nucleic acid level which distinguish among alleles of each group have become the focus of recent research. Current methods of high resolution typing include the following.

The Sequence Specific Oligonucleotide Probes ("SSOP") technique, as described in United States Patent No. 5,451,512 assigned to Hoffman-La Roche, Inc., uses a reverse dot blot format, wherein HLA-A probes are immobilized on a membrane, and the labelled target (patient sample) DNA is hybridized to the membrane-bound probe (as described in Saiki et al., 1989, *Proc. Natl. Acad. Sci.* 86:6230-6234). The pattern of hybridization to the probes on the dot-blot gives information regarding the HLA type of the individual. However, because hybridization is inherently not sufficiently specific to rule out minor differences in sequence between probe and patient sample, there is a possibility that the patient sample may contain an allelic variant which is not accounted for.

Another nucleic acid-based test is the Amplification Refractory Mutation System (ARMS) as described in the "HLA Class I SSP ARMS-PCR Typing Kit" Reference Manual, June 1995 edition, published by the Imperial Cancer Research Fund. This assay is based on the need for complementarity (matching) between the 3' end of an amplification primer and a target DNA sequence. Absent such matching, the primer will not function properly and no fragment will be amplified. Sequence information is deduced by determining, for various pairs of primers acting on target DNA from a patient sample, whether or not a fragment is successfully amplified. The accuracy of the technique is limited by the number of primer pairs tested and by the possibility that allelic variations exist in regions of DNA which lie between the primers.

In order to overcome the foregoing shortcomings, it has been proposed that typing be accomplished by direct DNA sequencing (Santamaria et al., "HLA Class I Sequence-Based Typing" Hum. Immunol. 37, 39-50 (1993); WO 9219771; US Pat. 5,424,184). However, while direct sequencing of a patient's Class I HLA locus may conceptually be the most accurate, such sequencing may require a time-frame unsuitable for clinical practice. The success of direct sequencing methods may be expected to rely upon the design of efficient protocols and relevant primer sequences.

Prior to the present invention, direct sequencing protocols have exhibited a number of disadvantages. For example, the method of Santamaria et al., *supra*, fails to provide sufficient information because it focuses on cDNA (exon) sequences which, in view of exon sequence diversity, offer a very limited selection of conserved primer hybridization sites. In addition, because the Santamaria sequencing primers hybridize within an exon, they do not provide information for DNA sequence upstream of the primer which is potentially decisive for distinguishing among alleles. Further, the sites disclosed were determined before the recent discovery of dozens of more alleles that now need to be considered in identifying HLA type.

Intron sequences could provide the preferred hybridization sites for amplification and sequencing primers for the HLA-A, HLA-B and HLA-C genes because they may provide the DNA sequence of the full exon. Intron sequences for an HLA Class I gene were disclosed at least as early as 1985 (Weiss et al Immunobiol 170:367-380, (1985)). Due to their substantial diversity, and the difficulties in sequencing, few intron sequences have been published subsequently.

A number of researchers have made limited use of intron based oligonucleotides for limited aspects of HLA Class I typing.

Blasczyk et al. (Tissue Antigens 1996: 47: 102-110) used exon based amplification primers to determine group specificity. After amplification, universal sequencing primers located in intron 2 were used to sequence the amplified fragment. The paper does not disclose any intron sequence motifs from intron 1 or 3 or the 5' untranslated region.

Cereb et al. (Tissue Antigens 1995: 45:1-11), undertook the

identification of intron sequences useful for locus-specific amplification primer sets for all Class I genes. These primer sets were designed to amplify all alleles of the same locus. No group specific amplification primers were sought or reported. Further, amplified fragments were characterized by SSOP and not by direct sequencing.

Johnston-Dow et al (Poster Presentation: 1995 ASHI Meeting, Dallas, TX) presented a system for direct sequence determination of HLA-A wherein degenerate exon based primers were used to amplify exons 1 to 5 of the genomic HLA-A DNA sequence. As in Cereb et al., supra, the degenerate primer pool was designed to amplify all alleles of the HLA-A locus. Group specificity was not sought or reported. Further, sequencing of the amplified fragment was obtained using a degenerate primer mix wherein primers hybridize to intron regions flanking exons 2 and 3.

A rational approach to typing of classical HLA Class I loci would provide a simplified series of steps for high resolution typing of each allele of each loci in a patient sample using intron based oligonucleotides. Further, this method would be able to identify new alleles without ambiguities.

An alternative method of intron based HLA Class I typing is the subject of previously filed US Patent Application Serial No. 08/pending (Atty Docket No. VGEN.P-037-US), assigned to an assignee of the present invention.

3. SUMMARY OF THE INVENTION

The present invention relates to materials and methods for high-resolution, nucleic acid-based typing of the three classical HLA Class I genes (comprising the loci HLA-A, HLA-B and HLA-C) in a patient sample. It is based, in part, on the discovery of group-specific sequence motifs, derived from the analysis of numerous patient samples, which include sequences of the 5' flanking region, intron 1, intron 2, and intron 3. Such sequence motifs may be used to design amplification primers which may be used to identify the HLA group or type of a subject. The invention is also based, in part, on the determination of numerous allele-specific sequences which may be used to confirm the precise allelic type of a subject.

The present invention provides for substantially purified nucleic acids which are capable of selectively hybridizing with group specific sequence motifs in untranslated regions of the HLA-A, HLA-B or HLA-C gene loci. Such nucleic acids, which may be comprised in a kit, may be used, alone or in conjunction with exon-based primers, to determine the group specificity of HLA-A, HLA-B, or HLA-C alleles contained in a patient sample and to identify the specific alleles present.

In particular embodiments, the present invention provides for methods of ascertaining the HLA Class I type of a subject which comprise performing a first amplification reaction which identifies the group type of the subject, and a second amplification reaction which produces allele-specific nucleic acids for sequencing.

3.1. DEFINITIONS

"Allele" means one of the alternative forms of the gene in question;

"Amplification" means the process of increasing the relative abundance of one or more specific genes or gene fragments in a reaction mixture with respect to the other genes. A method of amplification which is well known by those skilled in the art is the polymerase chain reaction (PCR) as described in United States Patents Nos. 4,683,194, 4,683,195 and 4,683,202, which are incorporated herein by reference. The PCR process involves the use of pairs of primers, one for each complementary strand of the duplex DNA (wherein the coding strand is referred to as the "sense strand" and its complementary strand is referred to as the "antisense strand"), that will hybridize at a site located near a region of interest in a gene. Chain extension polymerization (without a chain terminating nucleotide) is then carried out in repetitive cycles to increase the number of copies of the region of interest many times. The amplified oligonucleotides are then separated from the reaction mixture and used as the starting sample for the sequencing reaction. Gelfand et al. have described a thermostable enzyme, "*Taq* polymerase," derived from the organism *Thermus aquaticus*, which is useful in this amplification process (see United States Patent Nos. 5,352,600 and 5,079,352 which are incorporated herein by reference);

"Group" as used herein, refers to a subset of alleles of one loci, all of which share sequence features which distinguish them from other groups. For

example, serological group reactivity (in a lymphocytotoxicity assay) is the conventional basis for nomenclature of HLA alleles. The first two digits of an allele refer to the serological group; for example, the designation A*0201, A*0202, A*0217 all are members of the A2 group. Further, typically the nomenclature refers to the serological split group (e.g., A23 and A24 are serological splits of A9;

"Group-specific sequence motif" means a generally short, 1-25 nucleotide ("nt") sequence of nucleic acid which is found only in one or a few groups. Where a motif is shared by several groups in one region of the HLA locus, group-specific sequence motifs in other regions of the locus may serve as group-distinguishing features. The motif may share one or more nucleotides with the consensus sequence for the region;

"Haplotype" means the allele present on one chromosome;

"Heterozygote" means the presence of at least two different alleles of a gene;

"Homozygote" means the presence of a single species of allele of a gene;

"Locus" means a gene, such as HLA-A, HLA-B or HLA-C;

"Locus specific" means an event or thing associated with only one locus;

"Patient sample" means a sample collected from a patient in need of HLA typing which contains a sufficient amount and quality of nucleic acid (preferably DNA) for the performance of an amplification reaction. A nonlimiting example of a suitable source is peripheral blood lymphocytes, tissue (including cell cultures derived therefrom, mucosal scrapes, spleen and bone marrow;

"Primer" means a polynucleotide generally of 5-50 nucleotides length which can serve to initiate a chain extension reaction;

"Sequencing" or "DNA sequencing" means the determination of the order of nucleotides in at least a part of a gene. A well known method of sequencing is the "chain termination" method first described by Sanger et al., Proc. Nat'l Acad. Sci. (USA) 74(12): 5463-5467 (1977) (recently elaborated in EP-B1- 655506, and Sequenase 2.0 product literature (Amersham Life Sciences, Cleveland) incorporated herein by reference). Basically, in this process, DNA to be sequenced is isolated,

rendered single stranded, and placed into four vessels. In each vessel are the necessary components to replicate the DNA strand, which include a template-dependant DNA polymerase, a short primer molecule complementary to a known region of the DNA to be sequenced, and individual nucleotide triphosphates in a buffer conducive to hybridization between the primer and the DNA to be sequenced and chain extension of the hybridized primer. In addition, each vessel contains a small quantity of one type of optionally detectably labeled dideoxynucleotide triphosphate, *e.g.*, dideoxyadenosine triphosphate ("ddA"), dideoxyguanosine triphosphate ("ddG"), dideoxycytosine triphosphate ("ddC"), or dideoxythymidine triphosphate ("ddT"). In each vessel, each piece of the isolated DNA is hybridized with a primer. The primers are then extended, one base at a time to form a new nucleic acid polymer complementary to the isolated pieces of DNA. When a dideoxynucleotide is incorporated into the extending polymer, this terminates the polymer strand and prevents it from being further extended. Accordingly, in each vessel, a set of extended polymers of specific lengths are formed which are indicative of the positions of the nucleotide corresponding to the dideoxynucleic acid in that vessel. These sets of polymers are then evaluated using gel electrophoresis to determine the sequence.

"Specific hybridization" means hybridization of one strand of a nucleic acid to its complement.

"Target sequence" means the preferred site for specific hybridization of a primer; and

"Untranslated region" refers to a portion of an HLA locus which is not transcribed into RNA and eventually translated into protein. Examples of untranslated regions are the 5' and 3' flanking regions and intron sequences. For example, the 5' flanking region is neither transcribed nor translated, and intron sequences are transcribed but not translated.

4. DESCRIPTION OF THE FIGURES

FIGURE 1 is an illustration of the principle for an HLA class I sequencing strategy. Group-specific primers are used for PCR amplification, and universal primers located in the 2nd intron are used for sequencing, regardless of the amplified group. 5'FR= 5' flanking region; 5' UTR= 5' untranslated region (-1 to -23 from the ATG start codon in exon 1).

FIGURE 2A and 2B depict, in schematic form, a method of the invention in which a cocktail of HLA-A group specific primers is used to amplify target DNA contained in a patient sample. The products of amplification are then separated electrophoretically in an agarose gel, allowing the identification, by fragment mobility, of fragments corresponding to groups A2 and A3. Primers specific for groups A2 and A3 are then used to amplify duplicate samples of target DNA in separate reactions, to produce A2 and A3 fragments which may then be sequenced using universal sequencing primers. FIGURE 2C and 2D depict a strategy wherein group type specificity is determined by reaction of aliquots of genomic DNA in separate reactions with a panel of primer pairs.

FIGURE 3 depicts the nucleic acid sequences of the HLA-A 5' flanking region in various alleles, including a consensus sequence (SEQ ID NO:1) as well as the sequences for the following alleles: A*0101 (SEQ ID NO:2); A*0301 (SEQ ID NO:3); A*1101 (SEQ ID NO:4); A*1102 (SEQ ID NO:5); A*3001 (SEQ ID NO:6); A*3002 (SEQ ID NO:7); A*3004 (SEQ ID NO:8); A*0201-11 (SEQ ID NO:9); A*0215 (SEQ ID NO:10); A*0217 (SEQ ID NO:11); A*6801 (SEQ ID NO:12); A*6802 (SEQ ID NO:13); A*6901 (SEQ ID NO:14); A*2301 (SEQ ID NO:15); A*2402 (SEQ ID NO:16); A*2403 (SEQ ID NO:17); A*2404 (SEQ ID NO:18); A*2405 (SEQ ID NO:19); A*2407 (SEQ ID NO:20); A*2501 (SEQ ID NO:21); A*2601 (SEQ ID NO:22); A*3402 (SEQ ID NO:23); A*4301 (SEQ ID NO:24); A*6601 (SEQ ID NO:25); A*6602 (SEQ ID NO:26); A*6603 (SEQ ID NO:27); A*2901 (SEQ ID NO:28); A*2902 (SEQ ID NO:29); A*31012 (SEQ ID NO:30); A*3201 (SEQ ID NO:31); A*3301 (SEQ ID NO:32); A*3303 (SEQ ID NO:33); A*7401 (SEQ ID NO:34); A*7402 (SEQ ID NO:36); A*7403 (SEQ ID NO:37); and A*8001 (SEQ ID NO:38).

FIGURE 4 depicts the nucleic acid sequences of HLA-A intron 1 in various alleles, including a consensus sequence (SEQ ID NO:39) as well as the sequences for the following alleles: A*0101 (SEQ ID NO:40); A*0301 (SEQ ID NO:41); A*1101 (SEQ ID NO:42); A*1102 (SEQ ID NO:43); A*3001 (SEQ ID NO:44); A*3002 (SEQ ID NO:45); A*3004 (SEQ ID NO:46); A*0201 (SEQ ID NO:47); A*0202 (SEQ ID NO:48); A*0203 (SEQ ID NO:49); A*0204 (SEQ ID NO:50); A*0205 (SEQ ID NO:51); A*0206 (SEQ ID NO:52); A*0207 (SEQ ID NO:53); A*0208 (SEQ ID NO:54); A*0209 (SEQ ID NO:55); A*0210 (SEQ ID NO:56); A*0211 (SEQ ID NO:57); A*0215 (SEQ ID NO:58); A*0217 (SEQ ID NO:59); A*6801 (SEQ ID NO:60); A*6802 (SEQ ID NO:61); A*6901 (SEQ ID NO:62); A*2301 (SEQ ID NO:63); A*2402 (SEQ ID NO:64); A*2403 (SEQ ID NO:65); A*2404 (SEQ ID NO:66); A*2405 (SEQ ID NO:67); A*2407 (SEQ ID NO:68); A*2501 (SEQ ID NO:69); A*2601 (SEQ ID NO:70); A*3402 (SEQ ID NO:71); A*6601 (SEQ ID NO:72); A*6602 (SEQ ID NO:73); A*6603 (SEQ ID NO:74); A*4301 (SEQ ID NO:75); A*2901 (SEQ ID NO:76); A*2902 (SEQ ID NO:77); A*3101 (SEQ ID NO:78); A*3201 (SEQ ID NO:79); A*3301 (SEQ ID NO:80); A*3303 (SEQ ID NO:81); A*7401 (SEQ ID NO:82); A*7402 (SEQ ID NO:83); A*7403 (SEQ ID NO:84); and A*8001 (SEQ ID NO:85).

FIGURE 5 depicts the nucleic acid sequences of HLA-A intron 2 in various alleles, including a consensus sequence (SEQ ID NO:86) as well as sequences for the following alleles: A*0101 (SEQ ID NO:87); A*0201 (SEQ ID NO:88); A*0202 (SEQ ID NO:89); A*0203 (SEQ ID NO:90); A*0204 (SEQ ID NO:91); A*0205 (SEQ ID NO:92); A*0206 (SEQ ID NO:93); A*0207 (SEQ ID NO:94); A*0208 (SEQ ID NO:95); A*0209 (SEQ ID NO:96); A*0210 (SEQ ID NO:97); A*0211 (SEQ ID NO:98); A*0215 (SEQ ID NO:99); A*0217 (SEQ ID NO:100); A*6801 (SEQ ID NO:101); A*6802 (SEQ ID NO:102); A*6901 (SEQ ID NO:103); A*2501 (SEQ ID NO:104); A*2601 (SEQ ID NO:105); A*4301 (SEQ ID NO:106); A*6601 (SEQ ID NO:107); A*6602 (SEQ ID NO:108); A*6603 (SEQ ID NO:109); A*3402 (SEQ ID NO:110); A*2901 (SEQ ID NO:111); A*2902 (SEQ ID NO:112); A*3101 (SEQ ID NO:113); A*3201 (SEQ ID NO:114); A*3301 (SEQ ID NO:115);

A*3303 (SEQ ID NO:117); A*7401 (SEQ ID NO:118); A*7402 (SEQ ID NO:119); A*7403 (SEQ ID NO:120); A*2301 (SEQ ID NO:121); A*2402 (SEQ ID NO:122); A*2403 (SEQ ID NO:123); A*2404 (SEQ ID NO:124); A*2405 (SEQ ID NO:125); A*2407 (SEQ ID NO:126); A*0301 (SEQ ID NO:127); A*1101 (SEQ ID NO:128); A*1102 (SEQ ID NO:129); A*3001 (SEQ ID NO:130); A*3002 (SEQ ID NO:131); A*3004 (SEQ ID NO:132); and A*8001 (SEQ ID NO:133).

FIGURE 6 depicts the nucleic acid sequences of HLA-A intron 3 in various alleles, including a consensus sequence (SEQ ID NO:134) as well as sequences for the following alleles: A*0101 (SEQ ID NO:135); A*0301 (SEQ ID NO:136); A*1101 (SEQ ID NO:137); A*1102 (SEQ ID NO:138); A*3001 (SEQ ID NO:139); A*3002 (SEQ ID NO:140); A*3004 (SEQ ID NO:141); A*0201 (SEQ ID NO:142); A*0202 (SEQ ID NO:143); A*0203 (SEQ ID NO:144); A*0204 (SEQ ID NO:145); A*0205 (SEQ ID NO:146); A*0206 (SEQ ID NO:147); A*0207 (SEQ ID NO:148); A*0208 (SEQ ID NO:149); A*0209 (SEQ ID NO:150); A*0210 (SEQ ID NO:151); A*0211 (SEQ ID NO:152); A*0215 (SEQ ID NO:153); A*0217 (SEQ ID NO:154); A*6801 (SEQ ID NO:155); A*6802 (SEQ ID NO:156); A*6901 (SEQ ID NO:157); A*2301 (SEQ ID NO:158); A*2402 (SEQ ID NO:159); A*2403 (SEQ ID NO:160); A*2404 (SEQ ID NO:161); A*2405 (SEQ ID NO:162); A*2407 (SEQ ID NO:163); A*2501 (SEQ ID NO:164); A*2601 (SEQ ID NO:165); A*3402 (SEQ ID NO:166); A*4301 (SEQ ID NO:167); A*6601 (SEQ ID NO:168); A*6602 (SEQ ID NO:169); A*6603 (SEQ ID NO:170); A*2901 (SEQ ID NO:171); A*2902 (SEQ ID NO:172); A*3101 (SEQ ID NO:173); A*3201 (SEQ ID NO:174); A*3301 (SEQ ID NO:175); A*3303 (SEQ ID NO:176); A*7401 (SEQ ID NO:177); A*7402 (SEQ ID NO:178); A*7403 (SEQ ID NO:179); and A*8001 (SEQ ID NO:180).

FIGURE 7 depicts a phylogenetic tree of the 5' flanking and 5' untranslated regions of HLA-A.

FIGURE 8 depicts a phylogenetic tree of introns 1-3 of the HLA-A gene.

FIGURE 9 depicts a phylogenetic tree of introns 1-3 of the HLA-B gene.

FIGURE 10 depicts the results of amplification using group-specific

exon region primers to determine HLA-A group type, wherein the group specificity is determined to be 6601 and 3201 (see Table 7).

FIGURE 11 depicts the results of amplification using group-specific exon region primers to determine HLA-A group type, wherein the group specificity is determined to be 020x and 680x (see Table 8).

FIGURE 12 depicts the nucleic acid sequences of the first intron of HLA-B, including a consensus sequence (SEQ ID NO:246) as well as the sequences for the following alleles: B*0702 (SEQ ID NO:247), B*0801 (SEQ ID NO:248), B*1302 (SEQ ID NO:249), B*1401 (SEQ ID NO:250), B*1402 (SEQ ID NO:251), B*1501 (SEQ ID NO:252), B*1502 (SEQ ID NO:253), B*1505 (SEQ ID NO:254), B*1508 (SEQ ID NO:255), B*1510 (SEQ ID NO:256), B*1512 (SEQ ID NO:251), B*1513 (SEQ ID NO:258), B*1517 (SEQ ID NO:259), B*1525 (SEQ ID NO:260), B*1532 (SEQ ID NO:261), B*1801 (SEQ ID NO:262), B*1805 (SEQ ID NO:263), B*27052 (SEQ ID NO:264), B*27053 (SEQ ID NO:265), B*2707 (SEQ ID NO:266), B*3501 (SEQ ID NO:267), B*3502 (SEQ ID NO:268), B*3503 (SEQ ID NO:269), B*3701 (SEQ ID NO:270), B*3801 (SEQ ID NO:271), B*3901 (SEQ ID NO:272), B*3903 (SEQ ID NO:273), B*3906 (SEQ ID NO:274), B*4001 (SEQ ID NO:275), B*4002 (SEQ ID NO:276), B*4101 (SEQ ID NO:277), B*4102 (SEQ ID NO:278), B*4201 (SEQ ID NO:279), B*4402 (SEQ ID NO:280), B*4403 (SEQ ID NO:281), B*4501 (SEQ ID NO:282), B*4601 (SEQ ID NO:283), B*4701 (SEQ ID NO:284), B*4801 (SEQ ID NO:285), B*4901 (SEQ ID NO:286), B*5001 (SEQ ID NO:287), B*5101 (SEQ ID NO:288), B*5108 (SEQ ID NO:289), B*5201 (SEQ ID NO:290), B*5301 (SEQ ID NO:291), B*5401 (SEQ ID NO:292), B*5501 (SEQ ID NO:293), B*5601 (SEQ ID NO:294), B*5701 (SEQ ID NO:295), B*5801 (SEQ ID NO:296), B*5901 (SEQ ID NO:297), B*6701 (SEQ ID NO:298), B*7301 (SEQ ID NO:299).

FIGURE 13A-B. depicts the nucleic acid sequences of the second intron of HLA-B, including a consensus sequence (SEQ ID NO:300) as well as the following alleles: B*0702 (SEQ ID NO:301), B*0801 (SEQ ID NO:302), B*1302 (SEQ ID NO:303), B*1401 (SEQ ID NO:304), B*1402 (SEQ ID NO:305), B*1501(62) (SEQ ID NO:306), B*1505(62) (SEQ ID NO:307), B*1508(62) (SEQ ID

NO:308), B*1510(71) (SEQ ID NO:309), B*1513(77) (SEQ ID NO:310), B*1517(63) (SEQ ID NO:311), B*1525(62) (SEQ ID NO:312), B*1532(62) (SEQ ID NO:313), B*1801 (SEQ ID NO:314), B*2702 (SEQ ID NO:315), B*2704 (SEQ ID NO:316), B*27052 (SEQ ID NO:317), B*27053 (SEQ ID NO:318), B*2707 (SEQ ID NO:319), B*3501 (SEQ ID NO:320), B*3502 (SEQ ID NO:321), B*3503 (SEQ ID NO:322), B*3507 (SEQ ID NO:323), B*3508 (SEQ ID NO:324), B*3701 (SEQ ID NO:325), B*3801 (SEQ ID NO:326), B*3901 (SEQ ID NO:327), B*3903 (SEQ ID NO:328), B*3906 (SEQ ID NO:329), B*4001 (SEQ ID NO:330), B*4002 (SEQ ID NO:331), B*4101 (SEQ ID NO:332), B*4102 (SEQ ID NO:333), B*4201 (SEQ ID NO:334), B*4402 (SEQ ID NO:335), B*4403 (SEQ ID NO:337), B*4501 (SEQ ID NO:338), B*4601 (SEQ ID NO:339), B*4701 (SEQ ID NO:340), B*4801 (SEQ ID NO:341), B*4901 (SEQ ID NO:342), B*5001 (SEQ ID NO:343), B*5101 (SEQ ID NO:344), B*5108 (SEQ ID NO:345), B*5201 (SEQ ID NO:346), B*5301 (SEQ ID NO:347), B*5401 (SEQ ID NO:348), B*5501 (SEQ ID NO:350), B*5601 (SEQ ID NO:351), B*5701 (SEQ ID NO:352), B*5801 (SEQ ID NO:353), B*5901 (SEQ ID NO:354), B*6701 (SEQ ID NO:355), B*7301 (SEQ ID NO:356).

FIGURE 14A-E. depicts the nucleic acid sequences of the third intron of HLA-B, including a consensus sequence (SEQ ID NO: 357) as well as the following alleles: B*0702 (SEQ ID NO:358), B*0801 (SEQ ID NO:359), B*1302 (SEQ ID NO:360), B*1401 (SEQ ID NO:361), B*1402 (SEQ ID NO:362), B*1501 (SEQ ID NO:363), B*1502 (SEQ ID NO:364), B*1510 (SEQ ID NO:365), B*1513 (SEQ ID NO:366), B*1517 (SEQ ID NO:367), B*1525 (SEQ ID NO:368), B*1801 (SEQ ID NO:369), B*27052 (SEQ ID NO:370), B*27053 (SEQ ID NO: 371), B*3501 (SEQ ID NO:372), B*3502 (SEQ ID NO:373), B*3503 (SEQ ID NO:374), B*3701 (SEQ ID NO:375), B*3801 (SEQ ID NO:376), B*3903 (SEQ ID NO:377), B*3906 (SEQ ID NO:378), B*4001 (SEQ ID NO:379), B*4002 (SEQ ID NO:380), B*4101 (SEQ ID NO:381), B*4102 (SEQ ID NO:382), B*4201 (SEQ ID NO:383), B*4402 (SEQ ID NO:384), B*4403 (SEQ ID NO:385), B*4501 (SEQ ID NO:386), B*4601 (SEQ ID NO:387), B*4701 (SEQ ID NO:388), B*4901 (SEQ ID NO:389), B*5001 (SEQ ID NO:390), B*5101 (SEQ ID NO:391), B*5108 (SEQ ID NO:392), B*5201 (SEQ ID NO:393), B*5301 (SEQ ID NO:394), B*5401 (SEQ ID NO:395),

B*5501 (SEQ ID NO:396), B*5601 (SEQ ID NO:397).

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to compositions and methods which may be used to efficiently and accurately determine the HLA Class I type of a patient sample.

The present invention is based, in part, on the determination of group-specific sequence motifs in regions of HLA Class I loci. These motifs may be used to design oligonucleotides which may be used as group-specific primers in nucleic acid amplification reactions. The present invention is also based, in part, on the determination of the sequences of regions of a wide variety of alleles of HLA Class I loci; such sequences may be used to distinguish one allele from another. Sequences of regions including the 5' flanking region of HLA-A and introns 1, 2 and 3 of HLA-A are provided herein, and are set forth in Figures 3-6.

In general, the methods of the invention may be described as follows. Comparison of nucleotide sequences of an HLA locus among members of an HLA Class I group, which lie in either untranslated or exon regions, may be used to identify group-specific motif sequences. Identification of groups may be by establishing serological relationships or using phylogenetic information, as set forth in Figures 7-9. Based on the group-specific motif sequences, oligonucleotide primers may be designed, synthesized, and used to amplify a portion of the HLA locus. Oligonucleotides used in this manner are referred to herein as "group-specific primers" and, in particular, as "group-specific untranslated region primers" or "group-specific exon region primers", as the case may be.

In preferred nonlimiting embodiments of the invention, the primers correspond to untranslated regions of the HLA Class I locus ("group-specific untranslated region primers"). Such primers may be used in pairs, wherein each member of the pair hybridizes to an untranslated region lying on either side of at least one exon. For example, but not by way of limitation, primer pairs may be oligonucleotide pairs which hybridize to group-specific motifs in the 5' untranslated region and the first, second, or third intron; the first intron and the second or third

intron; or the second and third intron.

The group-specific primers may be used in several different methods according to the invention. In a first series of nonlimiting embodiments, the group-specific primers may be used in a diagnostic manner to identify which allelic groups are present in a patient sample. In a second series of nonlimiting embodiments, the group-specific primers may be used to amplify sufficient amounts of a particular allelic fragment which is then subjected to direct nucleotide sequencing using universal sequencing primers.

According to the first series of embodiments, the present invention provides for a method of determining the HLA Class I group type of a subject comprising (i) combining a group-specific primer pair with a target DNA sample from the subject under conditions such that primer-based amplification of the target DNA may occur; and (ii) determining whether a nucleic acid product is produced by the amplification; wherein the ability of a primer pair to produce a nucleic acid product is associated with a particular HLA group type. The group-specific primers may be group-specific exon region primers or group-specific untranslated region primers. In related embodiments the present invention provides for a method of determining the HLA Class I group type of a subject comprising (i) combining a plurality of group-specific exon region primer pairs with a target DNA sample from the subject under conditions such that primer-based amplification of the target DNA may occur; (ii) determining the size of the nucleic acid products of the amplification; and (iii) correlating the size of the product with the predicted size of a fragment associated with a particular HLA group type. The plurality of primers is referred to as an HLA "cocktail" (see Figures 1 and 2). These first methods may be used to provide useful diagnostic information. For example, group type determination may serve as a first level of comparison for a histocompatibility analysis, even without identification of the specific allele(s) involved. For example, if a potential donor and host are being evaluated for tissue transplantation, if it is found that their group types do not match, no further comparison may be necessary. If, alternatively, their types do match, further analysis, for example by direct sequencing, may be desirable.

According to the second series of embodiments, the present invention

provides for a method of determining the HLA Class I allelic type of a subject comprising (i) combining a group-specific oligonucleotide primer pair with a target DNA sample from the subject under conditions such that primer-based amplification of the target DNA may occur; (ii) collecting the nucleic acid product of the amplification; and (iii) determining the nucleic acid sequence of the product. The group-specific primer pair used may be determined based on the group type of the subject, as determined using the first method, described above. In preferred embodiments of the invention, group-specific untranslated region primers which span a region of the HLA locus containing allele-specific sequence may be utilized. If a subject is heterozygous, separate amplification reactions are performed for each group identified (*e.g.*, separate reactions to amplify fragment for group A2 and group A3; see Figure 2). Sequencing may be performed using universal sequencing primers which will operate irrespective of HLA group or allelic type.

A more detailed description of the invention follows. Most alleles of the classical HLA Class I gene loci (consisting of HLA-A, HLA-B and HLA-C) can be distinguished on the basis of exon 2 and 3 alone. In one non-limiting embodiment, a method of the invention takes advantage of this fact, and employs the strategy generally described in Figure 2, using the example of HLA-A. A genomic DNA sample is prepared from a patient sample according to well known techniques. Aliquots of the genomic DNA may then separately be reacted with a panel of group-specific exon region primer pairs (Figure 2C), wherein the successful amplification of a DNA fragment is associated with a particular group type. Alternatively, as depicted in Figure 2A), part of the sample may be treated with a cocktail of group-specific exon region primer pairs. Each primer pair in the cocktail will amplify only selected allelic groups because they specifically hybridize to group specific intron sequence motifs. Between them, under suitable polymerase chain reaction (PCR) conditions, the cocktail may amplify all known HLA-A groups, with each group specific amplification product having a different length. When reaction products are separated on an agarose gel the group(s) present in the patient sample may be identified by length.

Optionally, once the group specificity is determined, the direct

sequence of alleles may be determined for precise allelic identification. As illustrated in Figure 2 B), a further part of the patient sample DNA may be treated under PCR conditions with a pair of primers that are specific for the previously determined group; preferably such primers are group-specific untranslated region primers, which span greater distances of the locus. If two groups were detected, then two separate reactions are performed. At completion of the second amplification, the reaction products are sequenced using an intron based "universal primer" which hybridizes to an intron sequence which is conserved among all alleles of the locus. Though it is theoretically possible to use a sequencing primer which is specific for the amplified group only, it is found that using a universal primer simplifies the method and the preparation of a kit. Various universal sequencing primers are specifically provided herein (see *infra*) which hybridize, respectively, to intron sequences flanking the 5' end of exon 2, the 3' end of exon 2, the 5' end of exon 3 and the 3' end of exon 3.

The substantial advantage of the method of the invention is that the initial group specific amplification allows a PCR based separation of haplotypes in 95% of patient samples. The separation of the haplotypes is a major achievement of this protocol since it permits the resolution of cis/trans linkages of heterozygote sequencing results which cannot be achieved with other protocols. With the instant invention, a separation of the haplotypes may be achieved in serological heterozygous samples with the sequencing primer mixes ("PMs") described in Table 2 (*infra*) using group-specific amplification corresponding to the serological families. The selection of the PMs used for sequencing depends on the amplification patterns of the preceding PCR-SSP low-resolution typing. The primers are designed to work with a in a single cycle protocol including, but not limited to, a PCR protocol on a Perkin Elmer System 9600, maintaining typing capacities of the laboratory. All PCR products carry sufficient sequence information for a complete subtyping. This approach is superior to a typing system using a single pair of generic primers followed by direct sequencing or SSO hybridization, even if the amplification strategy is locus-specific. The substantial advantage of Sequence Based Typing (SBT) is the definition of the cis/trans linkage of sequence motifs. SBT after generic PCR amplification cannot define the cis/trans linkage of sequence motifs and therefore mimics oligotyping. The

rapidly growing number of newly identified alleles confirms that new alleles have arisen mainly from gene conversion events which have usually taken place between different alleles of the same locus. Newly identified alleles are not characterized by new sequence motifs, but by a new combination of already existing sequence motifs. From this observation it may be concluded that the amount of alleles at each locus may theoretically represent all possible combinations of known sequence motifs. Of course, some of them will fall victim to negative selection. Nevertheless, it can be expected that still an enormous amount of alleles are yet unidentified. PCR-SSP subtyping strategies using a restricted number of oligonucleotides which do not cover all possible sequence motifs suffer from this limitation. If the cis/trans linkage of the analyzed polymorphic regions is not defined some new alleles may be mistyped as a heterozygous combination of known alleles. This has consequences with respect to SBT strategies. An unambiguous typing result of SBT after generic PCR amplification is only unambiguous with regard to the presently known HLA sequence databank. However, with the detection of new alleles this result can become ambiguous over the course of time. This observation has already been made in PCR based DRB1 typing during the last five years and will probably also occur in PCR based class I typing. Considering the above points, the idea of the instant SBT approach is not only to identify the HLA-A, HLA-B and HLA-C subtypes, but to cover as many of the polymorphic sites as possible and to define the cis/trans linkage of the polymorphic sequence motifs. Typing results obtained with this method will remain unambiguous independently of the growing HLA sequence databank.

In general, group-specific primers are desirably designed to facilitate hybridization to their intended targets. It should be taken into account that homology between different groups, and indeed between group-specific motifs, may exist. Accordingly, in preferred embodiments of the invention, a primer may be designed such that it hybridizes to its group target under relatively stringent conditions. For example, one or more mismatched residues may be engineered into the 3' domain of the molecule. Further, the primer may be designed such that it differs from any naturally occurring or consensus sequence, but rather has mismatches inserted which serve to further reduce hybridization of the primer to target DNA of a group other

than the intended target group. Under certain circumstances, one or more mismatches may be introduced into the 5' end to destabilize internal hairpin loops; such changes are not generally expected to enhance the efficiency of the primer.

The following nucleic acid sequences may be comprised in group-specific untranslated region primers for HLA-A which are specific for the groups as indicated in Table 1. The sequences in Table 1 have the following sequence identifiers: II-210 is SEQ ID NO:35, and the remaining sequences II-230m through I3-282 have SEQ ID NOS S:181-202, respectively.

Table 1.

Designation	Sequence	N	Tm	Specificity	Position
II-210m S	5' ACC Cgg ggA gCC ggg CCT 3'	18	64°C	A10 et al.	73-92
II-220m S	5' ggC Agg TCT CAg CgA CTg 3'	18	60°C	A*01, 03, 11, 30	102- 119
II-226 S	5' CTC TgT ggg gAg AAg CAA C 3'	19	60°C	A802	29-47
II-221m11 S	5' ggg AgC ggC gCC ggg AC 3'	17	64°C	A*0301	77-93
II-209 S	5' gAA gCA Agg ggC CCg CCC 3'	18	64°C	A10 et al.	41-58
II-214m S	5' CgC CTg gCg ggg gGg CAA 3'	18	66°C	A*2301,24	54-71
II-223d S	5' gTg AgT gCg ggg TCg Tgg 3'	18	62°C	A19	1-19
II-225m S	5' gCC ggg Agg Agg gAC ggT 3'	18	64°C	A*30	85-103
II-237m14 S	5' ggC gCg CCC ggC ggg gA 3'	17	65°C	A*29	49-65
II-240 S	5' ggA ggA ggg TCg ggC ggA 3'	18	64°C	A*31,33	90-107
5'FL-243 S	5' AgT gTC TTC gCg gTC gCT C 3'	19	62°C	A*11	53-71
5'FR-257 S	5' CTC AgA TTC TCC CCA gAC g 3'	19	60°C	A all except A*11	6-24
5'FR-273 S	5' CATgCC gAg ggT TTC TCC CA 3'	20	64°C	A*28, 6602,6603	360- 380
BP202 S	5'CTg gCC CTg ACC CAg ACC A 3'	19	64°C	A*7401,7403	Exon 1, 49-68
BP203 S	5' CCT gAC CCA gAC CTg ggC A 3'	19	64°C	A*8001	Exon 1, 55-73

BP142 AS	5' CAGGTAT CTG CGGAGC CCG 3'	19	64°C	A*0101/*24	227- 245
I3-236 AS	5'gTC TgT CAg gAA gAgTCAgAA 3'	21	62°C	A*non02.28	584..+2
I3-239 AS	5'gTggAAAATTCTAgTCCCTgA A3'	22	62°C	A*multi, not A1,3,11,30,9	415- 436
I3-246 AS	5' AgATCT ACA ggC gAT CAg gA 3'	20	60°C	A*30	24-43
I3-247m6 AS	5' gCC AgC CCg ggA gTT CTA T 3'	19	62°C	A*01,11	38-54
I3-249 AS	5' CAg AgT CACTCTCTggTACAg 3'	21	62°C	All A, weak 59,70,92,J,E,G ,F	148- 168
I3-280m18 AS	5' gCg ATC gTC TTC CCg TCA C 3'	19	62°C	A*01,03,11,30	221- 239
I3-282 AS	5' AgAgTCACTCTC Tgg TACAgA 3'	21	62°C	A*8001	148- 168

The present invention provides for nucleic acid molecules comprising regions having the foregoing sequences or their functional equivalents.

"Functional equivalents" of a nucleotide sequence, as defined herein, refers to nucleotide sequences which, when contained in a nucleic acid molecule, retain the specificity of the disclosed sequence and/or hybridize to the complement of the disclosed sequence under stringent hybridization conditions (*e.g.*, 1 x SSC at 65°C).

In specific nonlimiting examples, oligonucleotides comprising the above sequences, or functional equivalents thereof which retain specificity, may be used in a PCR amplification reaction in the following pairwise combinations to generate group specific fragments of the lengths as indicated in Table 2.

Table 2.

Primer Mix No.	Name	Sense Primer	Antisense Primer	Size of Product	HLA-A specificity
1	1.1	I1-230m	BP142	785 bp	A*01
2	1.2	5'FR-257	I3-247m6	1068 bp	A*01
3	1.3	I1-230m	I3-247m6	870 bp	A*01,11
4	2	I1-226	I3-249	1056 bp	A*02
5	3	I1-221m11	I3-280m18	1078 bp	A*03
6	11	5'FL-243	I3-249	1229 bp	A*11
7	9	I1-214m	I3-249	1033 bp	A*23,24
8	10.1	I1-210m	I3-236	1450 bp	A*10
9	10.2	I1-210m	I3-249	1014 bp	A*10,68,69
10	28	5'FR-273	I3-249	1537 bp	A*68,69,6602,6603
11	19.1	I1-223d	I3-239 or I3-249	1084 bp	A*29,31,32,33,74
12	19.2	I1-240	I3-249	996 bp	A*31,33
13	29	I1-237m14	I3-249	1037 bp	A*29
14	30	I1-225m	I3-249	1000 bp	A*30
15	74	BP202 (Exon 1)	I3-249	1109 bp	A*7401,7403
16	80	BP203	I3-282	1103 bp	A*8001 (untested)

The following nucleic acid sequences may be comprised in group-specific exon region primers for HLA-A which are specific for the groups as indicated in Table 3 (sense primers) and Table 4 (antisense primers). The sequences in Table 3, primer numbers 85, 118, 120, 123, 127, 129, 134, 137, 140, 160, 167, 175, 193 and 202, have SEQ ID NOS:203-216, respectively. The sequences in Table 4, primer numbers 98, 115, 116, 117, 126, 133, 135, 136, 138, 142, 144, 145, 152, 153, 154, 155, 161, 165, 168, and 180, have SEQ ID NOS:217-236, respectively, and primer number 119 has SEQ ID NO:245. The present invention provides for nucleic acid molecules comprising regions having the foregoing sequences or their functional equivalents. They may, in specific nonlimiting examples, be used in pairs as set forth in Table 5.

Table 3.

Primer Number	Localization		Sequence
85	Exon 2	-14 - 5	5' CTC CTC gTC CCC Agg CTC T 3'
118	Exon 2	6 - 19	5' TCC ATg Agg TAT TTC TAC ACC 3'
120	Exon 3	-6 - 12	5' ggC CAg gTT CTC AgA CCA 3'
123	Exon 2	36 - 53	5' CCC ggC CCg gCA gTg gA 3'
127	Exon 3	1- 20	5' gTT CTC ACA CCA TCC AgA Tg 3'
129	Exon 3	4 - 25	5' TCA CAC CCT CCA gAT gAT gTT 3'
134	Exon 3	63 - 80	5' ggg TAC CAg CAg gAC gCT 3'
137	Exon 2	9 - 29	5' TCC ATg Agg TAT TTC ACC ACA 3'
140	Exon 3	-1 - 20	5' ggT TCT CAC ACC ATC CAg ATA 3'
160	Exon 3	1 - 20	5' gTT CTC ACA CCA TCC AgA gg
167	Exon 2	54 - 71	5' gAg CCC CgC TTC AAC gCC 3'
175	Exon 3	63 - 71	5' CTT CCT CCg Cgg gTA TgA A 3'
193	Exon 2	167 - 184	5' gCC ggA gTA TTg ggA CCg 3'
202	Exon 1	49 - 67	5' CTg gCC CTg ACC CTg ACC A 3'

Table 4. Antisense Primers

Primer Number	Localization		Sequence
98	Exon 2	226 - 243	5' gCA ggg TCC CCA ggT CCA 3'
115	Exon 3	195 - 213	5' CCT CCA ggT Agg CTC TCA A 3'
116	Exon 3	195 - 213	5' CCT CCA ggT Agg CTC TCC A 3'
117	Exon 3	195 - 213	5' CCT CCA ggT Agg CTC TCT g 3'
119	Exon 2	184 - 203	5' CTT CAC ATT CCg TgT CTC CT 3'
126	Exon 3	212 - 230	5' CCA CTC CAC gCA CgT gCC A 3'
133	Exon 2	229 - 246	5' ggA gCg CgA TCC gCA ggC 3'
135	Exon 3	216 - 234	5' ggA gCC ACT CCA Cgg ACC g 3'
136	Exon 3	216 - 233	5' gAg CCA CTC CAC gCA CTC 3'
138	Exon 2	186 - 206	5' ggC CTT CAC ATT CCg TgT gTT 3'
142	Exon 3	228 - 246	5' CAg gTA TCT gCg gAg CCC g 3'
144	Exon 2	165 - 184	5' Tgg TCC CAA TAC TCA ggC CT 3'
145	Exon 2	226 - 243	5' gCA ggg TCC CCA ggT TCg 3'

152	Exon 3	163 - 179	5' ggg CCg CCT CCC AgT TgT 3'
153	Exon 2	179 - 197	5' TCT gTg AgT ggg CCT aCA CA 3'
154	Exon 2	184 - 204	5' CCT TCA CAT TCC gTg TCT gCA 3'
155	Exon 3	216 - 233	5' gAg CCA CTC CAC gCA CgT 3'
161	Exon 2	209 - 228	5' CCA CTC ggT CAg TCT CTg AC 3'
165	Exon 3	105 - 124	5' gAg CgCA ggT CCT CgT TCA A 3'
168	Exon 2	198 - 217	5' gTC TgT gAg Tgg gCC aTC AT 3'
180	Exon 2	12 - 31	5' CAg CCA TAC ATC CTC Agg AC 3'

Table 5.

Group-specific exon pairs

Primer mix No.	Name	Sense Primer	Antisense Primer	Size of Product	HLA-A specificity
1	1	140	142	247 bp	A*0101,0102,8001
2	2	85	98	256 bp	A*0201-0220
3	3	140	126	230 bp	A*0301,0302,0303
4	36	167	168	164 bp	A*0101,3601
5	11	118	119	195 bp	A*1101-1103
6	23	129	115	209 bp	A*2301
7	24	129	116 + 117	209 bp	A*2402-2411
8	10.1	160	135	233 bp	A*2501,2601-2603,2605.4301,6601
9	25	118	233	238 bp	A*2501,2502
10	26	118	145	235 bp	A* 2601,2602, 2604,4301
11	34	134	155	171 bp	A*3401,3402
12	6602	134	136	240 bp	A*6602,6603
13	10.2	118	161	222 bp	A*11,34,6601,6602,68011,6802,6901
14	43	118	154	196 bp	A*4301
15	68	120	152	185 bp	A*68011,68012,6802,6803
16	69	193	180	375 bp	A*6901
17	19	127	165	124 bp	A*2901,2902,31012,3201,3301-3303 A*7401-7403
18	29	137	145	236 bp	A*2901-2902

19	30	175	115 + 116	162 bp	A*3001-3004
20	31	167	144	176 bp	A*31012
21	32	167	133	159 bp	A*3201,3202,2501,2502
22	33	137	138	198 bp	A*3301-3303
23	74	202	153	370 bp	A*7401,7403
24	80	140	136	234 bp	A*8001

In general, the foregoing group-specific primers may be modified by addition, deletion, or substitution of bases, to produce functionally equivalent primers with the substantially the same specificity, that is to say, such that the group specific polymorphism(s) are not removed. Such modifications may be constrained by several parameters. First, exact matching at the 3' end is particularly important for primer extension. Preferably, at least 5 nt are complementary to target DNA. When the exactly conserved region is short, for example, less than 10 nt, it is not advisable to change the primer sequences. The primer is preferably less than 50% G or C. Also, the primers should be designed to avoid specific hybridization with pseudogenes or non-classical HLA Class I loci. In the examples which follow, the melting temperature of all primers used was about 62C to ensure uniform amplification conditions.

For sequencing purposes, the following nucleic acid sequences are sequences which hybridize to all alleles of the indicated loci, in the locations indicated (and hence are referred to as universal sequencing primers). The primers in Table 6 are assigned consecutively SEQ ID NOS:237-244.

Table 6.

Designation	Sequence	Location	Melting Temp.
5'-Ex2(Aw3)	5' GCG CCG GGA GGA GGG TC 3'	Int-1	58-62°C
3'-Ex2	5' ATC TCG GAC CCG GAG ACT 3'	Int-2	58°C
5'-Ex3	5' GTT TCA TTT TCA GTT TAG GCC A 3'	Int-2	60°C
3'-Ex3(Aw6)	5' CGG GAG ATC TAC AGG CGA TCA GG 3'	Int-3	58-62°C
5'-Ex2(Aw3)	5' GCG CCG GGA GGA GGG TC 3'	Int-1	58-62°C
3'-Ex2	5' GTC GTG ACC TGC GCC CC 3'	Int-2	58-62°C
5'-Ex3	5' GGG CGG GGC GGG GCT CGG G 3'	Int-2	58-62°C
3'-Ex3(Aw6)	5' CGG GAG ATC TAC AGG CGA TCA GG 3'	Int-3	58-62°C
5'-Ex2 (Aw3)	5' GCG CCG GGA GGA GGG TC 3'	Int-1	58-62°C
3'-Ex2(ABCw1)	5' GGT CGT GAC CT(T/C)CGC CCC 3'	Int-2	58-62°C
5'-Ex3(ABCw2)	5' CCC GGT TTC ATT TTC 3'	Int-2	58-62°C
3'-Ex2(Aw6)	5' CGG GAG ATC TAC AGG CGA TCA GG 3'	Int-3	58-62°C

The foregoing three groups of primers include 5' and 3' primers for sequencing across exons 2 and 3, respectively.

The selection of suitable universal sequencing primers is constrained by a variety of rules including the following. Sequencing primer hybridization sites must lie within the fragment amplified by the group specific amplification primers. All primers are desirably selected to provide informative sequence and not start too far downstream of useful sequence. Preferred primers hybridize to conserved sites near the exon/intron boundaries.

Direct sequencing of the 2nd and 3rd exon may be performed from either the 5' or 3' end using the primers of Table 6 *supra* which are located in conserved regions of the 1st, 2nd and 3rd intron as indicated. These conserved regions were found to be identical in all samples investigated, regardless of the amplified group.

An important issue of direct sequencing for HLA class I genes is the generation of a specific PCR product, which is rather complicated due to the

extensive sequence homologies between the different HLA class I loci including several pseudogenes. If an adequate PCR product has been generated, any sequencing chemistry should be applicable.

In the normal case, since group specific amplifications take place before sequencing, only one allele at a time is sequenced, resulting in unambiguous homozygous sequencing results. In these cases alleles are relatively easy to identify, even without software.

However, in about 5% of cases, both alleles come from the same group, but the sequence results show heterozygosity. In practice, when viewed by a fluorescence-detecting system, the sample appears as a normal sequence of bases with, sporadically, two bases at one site, each with half the peak height. This result flows from the high degree of similarity shared among all alleles of each HLA gene; sequence heterozygosity flows from base substitutions. The laborious task of determining which alleles are present in the test sequence may be simplified using computer analysis. A software program called GeneLibrarian developed by Visible Genetics, the assignee of the present application, rapidly compares the test sequence to a database which includes all possible homozygote and heterozygote combinations of the alleles. The program identifies those stored sequences that are closest matched to the test sequence. The operator can then determine which allelic pair is in the test sample. If no allelic pair shows an exact match, the software allows the operator to review the test sequence to determine if errors in base-calling or other artifacts are interfering with the analysis.

The order of sequencing reactions may be selected by the operator. Each exon of each locus may be sequenced on the sense strand or anti-sense strand. A preferred method is to obtain sequence from one strand from each exon. If the results contain ambiguities, then the amplicon is re-sequenced using the other primer for the same exon. The availability of both sequencing primers provides redundancy to ensure robust results.

In some cases, it may be advantageous to employ an equimolar mixture of 2 or more oligonucleotide species. Mixtures of oligonucleotides may be selected such that between them they will effectively prime the sequencing reactions for all

alleles of the locus at the same site.

In an alternative technique, instead of using dye terminators, a dye-labelled primer may be employed. In this case, the selected sequencing primers is labelled on the 5' end with a detectable label, using phosphoramidite or NHS/dye ester techniques well known in the art. The label selected depends on the detection instrument employed. The label for use with an OpenGene System (Visible Genetics Inc., Toronto, ON) is the fluorophore Cy5.5 (Amersham Life Sciences, Cleveland OH). Fluorescein-isothio-cyanate may be used for detection with the ALF Automated Sequencer (Pharmacia, Piscataway NJ). In this method, which is well known to one skilled in the art, the sequencing reaction mixture is changed slightly to include only one ddNTP per reaction mixture. For detection of reaction products, the sample may be mixed with an equal volume of loading buffer (5% ficoll plus a coloured dye). 1.5 ul of these samples may be loaded per lane of a MicroCel electrophoresis cassette loaded in a MicroGene Blaster automated DNA sequencer (Visible Genetics Inc., Toronto). The sample may be electrophoresed and read.

Results may be displayed and analyzed with GeneObjects software. The sequence of bases may be determined, and the HLA allele to which the sequence corresponds may then be identified. This process may be performed for each locus (HLA-A, HLA-B, HLA-C) and the results may then be reported to the patient file.

It is well known in the art that different variations of sequencing chemistry may be employed with different automated DNA sequencing instruments. Single dye instruments, such as the OpenGene System (Visible Genetics Inc., Toronto), the ALF Express (Pharmacia, Uppsala, Sweden) or the Li-Cor 4000L (Lincoln City, Nebraska) generally use dye-labeled primers. In these systems a single chain termination sequencing reaction mixture is run per lane.

Multi-dye sequencers, such as the Prism 377 (applied Biosystems, Inc., Foster City, California) detect multiple dyes in a single lane. This technology conveniently employs dye-terminator chemistry, where the chain-terminating nucleotides are themselves labeled with fluorophores (see United States Patent No. 5,332,666, to Dupont de Nemours and Co.). In this case, the reaction products carrying four different labels may be run in a single lane.

Either single dye or multi-dye chemistry may be employed according to the present invention, along with other sequencing chemistries. Additional methods for reducing the numbers of reactions required to obtain detailed sequence information from the classical HLA Class I loci are disclosed in commonly owned United States Patent Applications USSN 08/577,858 (for single-track sequencing) and USSN 08/640,672 and 08/684,498 (for single-tube sequencing).

Directly analogous methods may be used to determine the HLA-B type of an individual. As with the HLA-A gene, the second and third exon of the HLA-B gene are polymorphic, and therefore provide for sequencing based typing strategies. A list of primers, together with their sequence, length, and localization, is provided in Table 7 below. The primers in Table 7 are assigned consecutively SEQ ID NOS:398-435.

TABLE 7
HLA-B PCR-SBT primer sequences
A. Amplification primers

Primer	Orient.	Sequence	N	Tm	Localization
E1-B121m17	S	5' CCA CCT gCT gCT CTC ggg A 3'	19	64	Exon 1, 27..45
E1-B129	S	5' CCT CCT gCT gCT CTC ggC 3'	18	62	Exon 1, 27..44
E1-B130	S	5' CTg CTg CTC Tgg ggg gCA 3'	18	62	Exon 1, 31..48
E1-B136	S	5' gAg ATg Cgg gTC ACg gCA 3'	18	60	Exon 1, -3..15
E1-B182	S	5' CTg ACC gA ₉ ACC Tgg gCT 3'	18	60	Exon 1, 55..72
I1-B145	S	5' Agg Agg gTC ggg Cgg gTT 3'	18	62	Intron 1, 90..108
I1-B154m	S	5' ggg TCT CAg CCC CAC CTT 3'	18	60	Intron 1, 114..121
I1-B167	S	5' gAg ggA AAT ggC CTC TgC C 3'	19	62	Intron 1, 17..35
I1-B168	S	5' Cgg ggg CgC Agg ACC TgA 3'	18	64	Intron 1, 59..76
I1-B169	S	5' gCg CCg ggA ggA ggg TCT 3'	18	64	Intron 1, 83..100
I1-B170	S	5' gCC TCT gTg ggg Agg AgA 3'	18	60	Intron 1, 27..44
I1-B171	S	5' gCC TCT gTA ggg Agg AgC A 3'	19	62	Intron 1, 27..45
I1-B170	S	5' gTC ggg Cgg gTC TCA gCT 3'	18	62	Intron 1, 97..114
I1-B173	S	5' Cgg ggg ACC gCg CCg gT 3'	17	64	Intron 1, 73..90
I1-B174	S	5' ggT CTC AgC CCC TCC TCA 3'	18	60	Intron 1, 105..122
I1-B175	S	5' gTg gAg TgC ggg gTC ggC 3'	18	60	Intron 1, -5..12
I1-B326	S	5' gTg AgT gCg ggg TCg gC 3'	17	60	Intron 1, 1..17
I1-B331	S	5' gAC CgC Agg Cgg ggg CT 3'	17	62	Intron 1, 50..66
I1-B346	S	5' TCT CAg CCC CTC CTC gCT 3'	18	60	Intron 1, 107..124
I3-B126	AS	5' gCC ATC CCC ggC gAC CTA T 3'	19	64	Intron 3, 36..54
I3-B147	AS	5' ggg ACC CCT gAT CAC TAT C 3'	19	60	Intron 3, 220..238
I3-B164	AS	5' ggC CCT CAg Agg AAA CTC g 3'	19	62	Intron 3, 134..152
I3-B165	AS	5' Agg CCT gAg Agg AAA AgT CAT 3'	21	62	Intron 3, 272..292
I3-B166	AS	5' Agg CgC TTT gCA TCT CTC ATA 3'	21	62	Intron 3, 535..555
I3-B187	AS	5' gAT CAg TAT TCT Agg gAC TgA 3'	21	60	Intron 3, 209..229
I3-B212	AS	5' gAA Tgg ACA ggA CAC CTg gT 3'	20	62	Intron 3, 481..500

TABLE 7 HLA-B PCR-SBT primer sequences A. Amplification primers					
Primer	Orient.	Sequence	N	Tm	Localization
I3-B305	AS	5' TCA TgC CAT TCT CCA TTC AAC 3'	21	60	Intron 3, 106..126
I3-B319	AS	5' CTA ggg ACT gTC TTC CCC TA 3'	20	62	Intron 3, 200..219
I3-B320	AS	5' CgC TgA TCC CAT TTT CCT CT 3'	20	60	Intron 3, 69..88
I3-B321	AS	5' CAg AgA ACA Agg CCT gAg AA 3'	20	60	Intron 3, 282..301
I3-B323	AS	5' AAC CCA gAC ACC AgC ggA T 3'	19	60	Intron 3, 443..463
I3-B332	AS	5' ggA CTT CTg CTC CTg ATC TA 3'	20	60	Intron 3, 363..382
I3-B335	AS	5' gAg gCC ATC CCg ggC gAT 3'	18	62	Intron 3, 40-57
I3-B337	AS	5' ggA AAg TTC gAg TCT CTg AgT 3'	21	62	Intron 3, 392..412
I3-B342	AS	5' CTC ATg CCA TTC TCC ATT CC 3'	20	60	Intron 3, 108..127
I3-B347	AS	5' TgA CCA gCC TgA gAA Tgg g 3'	19	60	Intron 3, 494..512
I3-B348	AS	5' AAC Agg gAC TTC TgC TCC C 3'	19	60	Intron 3, 369..387
I3-B349	AS	5' ggC CTg AgA ggA AAA gTC AC 3'	20	62	Intron 3, 272..291

Suitable primer mixes for HLA-B typing are set forth in Table 8 below.

TABLE 8 HLA-B PCR-SBT primer mixes					
Primer No. Name	Mix	Sense primer	Antisense primer	Size of product	HLA-B specificity
1	7	I1-B174	I3-B305	943bp	0702-0708,4801-4803,8101
2	8	I1-B167	I3-B323	1368b	0801-0804,4201
3	13	I1-B175	I3-B319	1145bp	1301-1304
4	14	I1-B145	I3-B321	1132bp	1401,1402
5	15	E1-B121m17	I3-B147	1204bp	1501-1537,4601
6	18	I1-B154m	I3-B164	960bp	1801-1805
7	27	E1-B182	I3-B349	1231bp	2701-2711,4002-4006, 4008, 4009, 4701

TABLE 8					
HLA-B PCR-SBT primer mixes					
Primer No. Name	Mix	Sense primer	Antisense primer	Size of product	HLA-B specificity
8	35	I1-B168	I3-B212	1363bp	3501-3521, 5101-5109, 5201, 5301, 5302, 5801, 5802, 7801, 7802
9	37	I1-B326	I3-B165	1213bp	1801-1805, 3701, 3702
10	16	I1-B167	I3-B320	993bp	3801, 3802, 3901-3912, 6701, 1401, 1402
11	60	I1-B172	I3-B342	952bp	4001, 4007, 4010
12	41	I1-B172	I3-B323	1288bp	4101, 4102
13	42	I1-B174	I3-B323	1280bp	4201, 4202
14	44	I1-B170	I3-B126	1323bp	4402, 4410
15	45	I1-B326	I3-B348	1307bp	4501
16	47	I1-B331	I3-B332	1254bp	4701, 4702
17	48	I1-B174	I3-B332	1199bp	4801-4803
31	49	I1-B326	I3-B337	1332bp	4901
18	50	I1-B326	I3-B187	1155bp	5001, 5002
19	22	I1-B169	I3-B166	1394bp	5401, 5501-5505, 5601-5603, 5901
20	57	I1-B171	I3-B347	1407bp	5701-5704
21	73	I1-B173	I3-B335	909bp	7301
22	78	I1-B168	I3-B212	1363bp	7801, 7802
23	82	I1-B346	I3-B126	868bp	8201
24	Multi I1	I1-B326	I3-B126	975bp	most 15, 1801-1805, 2701-2711, 4001-4010, 4101, 4102, 4501, 4601, 4901, 5001, 5002, 5701-5704
25	Multi I2	I1-B167	I3-B126	959bp	0702-0708, 0801-0804, 1401, 1402, 3801, 3802, 3901-3912, 4201, 4202, 4801-4803, 6701, 7801, 8101

TABLE 8					
HLA-B PCR-SBT primer mixes					
Primer No. Name	Mix	Sense primer	Antisense primer	Size of product	HLA-B specificity
26	Multi I3	I1-B168	I3-B126	917bp	0702-0708,0801-0804,1401,1402, 3501-3521,3801,3802,3901-3912, 4201,4202,4801-4803,5101-5109, 5201,5301,5302,5801,5802, 6701, 8101
27	Multi E1	E1-B129	I3-B126	1022bp	0702-0708,0801-0804,1401,1402, 3801,3802,3901-3912,4001,4007, 4010,4101,4102,4201,4202,4501, 4801-4803,4901,5001,5002,6701
28	Multi E2	E1-B130	I3-B126	1018bp	1301-1304,1801-1805,2701-2711, 3501-3521,3701,3702,4002-4006, 4008,4009,4402-4410,5101-5109, 5201,5301,5302,5701-5704,5801, 5802,7801,7802,8101
29	Multi E3	E1B-182	I3-B126	994bp	1801-1805,2701-2711,3701,3702, 4002-4006,4008,4009,4701
30	Multi E4	E1B-136	I3-B126	1051bp	4001,4007,4010,4101,4102,4501, 4901,5001,5002,5401,5501-5505, 5601-5603,5701-5704,5901

Sequencing primers suitable for HLA-B typing are set forth in Table 9,

below.

TABLE 9

B. Sequencing primers

Bseq2	AS	5' ggA TCT Cgg ACC Cgg AgA CTC g 3' 22 74°C Intron 2, 70..91 Mismatch for B*7301 at Pos. 9 and 10 from 3' end For Sequencing of HLA-B exon 2
Bseq3	S	5' ACC Cgg TTT CAT TTT CAg TTG 3' 21 60°C Intron 2,153..173 For Sequencing of HLA-B exon 3
Bseq3AB	S	5' TTT ACC Cgg TTT CAT TTT CAg TT 3' 23 62°C Intron 2,150..172 For Sequencing of HLA-A and B exon 3 Mismatch for B*7301 at Pos. 8 and 9 from 3' end Mismatch for A*8001 at Pos. 19 from 3' end
HLAB3X3.SEQ		5'TCC CCA CTG CCC CTG GTA 18 55°C Intron 3, 2-19 (also BC33, 3IN3BC02) No requirement for DEAZA
HLAB5X3.SEQ		5'GGK CCA GGG TCT CAC A 16 55°C Intron 2, 258- (also BC5X3INEX) Exon 3, 9 Requirement for DEAZA
HLAB3X2.SEQ		5'ATC TCG GAC CCG GAG ACT 18 60°C Intron 2, 78-98 (also A seq3) Requirement for DEAZA
HLAB5X2.SEQ		5'TCC CAC TCC ATG AGG TAT TTC 21 55°C Exon 2, 3-23 (also ABC25, SPE2, SPE2) No requirement for DEAZA

The primers in Table 9 are assigned consecutively SEQ ID NOS:436-442.

The protocol described in working example 8, *infra*, may be used to accomplish HLA-B typing using the foregoing materials.

The nucleic acids described above may be comprised in a kit for use in practicing the methods of the invention. In addition to the group-specific primers and primer pairs disclosed, such kits may further comprise buffers, reagents, and enzymes such as, amplification enzymes including but not limited to, *Taq* polymerase. In

specific, non-limiting embodiments, the kit may comprise group-specific exon region primers (for example, as a "cocktail" comprising a plurality of primers) as well as group-specific untranslated region primers; such primers may be contained in individual tubes.

In a specific, nonlimiting embodiment of the invention, the following method may be used to perform allele typing, here exemplified for HLA-B but, depending on the choice of primers, applicable to HLA-A as well. The following reagents may be used: 2.5 mM deaza dNTP Mix (2.5 mM dATP, 2.5 mM dCTP, 2.5 mM dTTP, 1.25 mM dGTP, 1.25 mM 7-DEAZA dGTP); 166 mM ammonium sulphate (Sigma BioSciences); 100% DMSO; PCR primers (e.g., pairs selected from Table 8); genomic DNA control (60 ng/ μ l); Sequencing Buffer (260 mM Tris-HCl, pH 8.3, 39 mM $MgCl_2$); 300:1 deaza terminators, including deaza A terminator (750 μ M dATP, 750 μ M dCTP, 560 μ M dGTP, 750 μ M dTTP, 190 μ M 7-deaza dGTP, 2.5 μ M ddATP), deaza C terminator (750 μ M dATP, 750 μ M dCTP, 560 μ M dGTP, 750 μ M dTTP, 190 μ M 7-deaza dGTP, 2.5 μ M ddCTP), deaza G terminator (750 μ M dATP, 750 μ M dCTP, 560 μ M dGTP, 750 μ M dTTP, 190 μ M 7-deaza dGTP, 2.5 μ M ddGTP) and deaza T terminator (750 μ M dATP, 750 μ M dCTP, 560 μ M dGTP, 750 μ M dTTP, 190 μ M 7-deaza dGTP, 2.5 μ M ddTTP); Sequencing Primers 5x2.seq, 3x2.seq, 5x3.seq, 3x3.seq (see, e.g., Table 9); Thermosequencase 32 U μ l (e.g., Thermosequencase cycle sequencing core kit, Amersham LifeScience, Product No. US 79610); Enzyme Dilution Buffer (10 mM Tris-HCl, pH 8, 1 mM 2-ME, 0.5% (v/v) Tween-20, 0.5% (v/v) NP-40; e.g., from Amersham LifeScience); Pink Loading Dye (Amersham); 10X PCR Buffer II (10 mM Tris-HCl, pH 8.3; 500 mM KCl); *Taq* DNA polymerase (e.g., Perkin Elmer or Roche); 25 mM $MgCl_2$; molecular grade water, and mineral oil (to prevent evaporation if a thermocycler without a heated lid is used). Apparatus used in the method may include a thermocycler (e.g., PE 9600 or MJ PTC) wherein the ramping time is adjusted to 1°C/sec, and tubes and trays supplied by the manufacturer of the thermocycler, wherein the use of trays and tubes fabricated from polypropylene rather than polystyrene is preferred.

First, according to the specific embodiment referred to in the preceding paragraph, the following HLA Locus Amplification Protocol may be used. Reagents

(except enzyme) may be thawed at room temperature, vortexed, and microfuged briefly, and placed on ice prior to use. Enzyme may be removed from the freezer when needed. On ice, the following master mix may then be prepared by combining, in the following order, (quantities provide for one 25 μ l reaction): molecular grade water 7.75 μ l; 10X PCR Buffer II (without $MgCl_2$) 2.5 μ l; 2.5 mM deaza dNTP Mix 2.0 μ l; 25 mM $MgCl_2$ 1.5 μ l; 100% DMSO 2.5 μ l; 166 mM Ammonium Sulphate 2.5 μ l; PCR primers 1.0 μ l; and 5U/ μ l *Taq* polymerase 0.25 (pipet gently up and down to mix). The master mix (which has a volume of 20 μ l) may then be introduced into a labelled 0.2 ml thin-walled amplification tube, and 5 μ l of 60 ng/ μ l genomic DNA may be added to produce a final concentration of 300 ng of DNA per reaction. The resulting reaction mixture may then be subjected to the following cycles in a thermocycler to result in amplification:

- (1) denaturation at 94°C for 5 minutes, cycle 1X with
- (2) denaturation at 94°C for 30 seconds;
- (3) annealing at 63°C for 30 seconds, cycle 35X with
- (4) extension at 72°C for 60 seconds;
- (5) extension at 72°C for 5 minutes, cycle 1X; and
- (6) soak at 4°C, cycle 1X.

To analyze the resulting amplification product, a 1% agarose gel containing ethidium bromide may be prepared, and 4 μ l of the PCR product may be loaded on the gel. Samples may then be run into the gel electrophoretically, along with size markers, and the size of the fragment may be compared with the size of the expected product (see, for example, Table 8).

The resulting amplification product may then be sequenced as follows. Four .2 ml thin-walled tubes may be placed on ice and labelled A, C, G and T, respectively. Three microliters each of deaza A, C, G and T terminators may be introduced into the appropriately labeled tube. Thermosequenase enzyme may then be diluted 1/10 in a separate tube by combining 1 μ l of thermosequenase with 9 μ l of enzyme dilution buffer, on ice. In a separate .5 ml tube, on ice, the following may be combined to form a master sequencing mix: Sequencing Buffer 2.5 μ l; Sequencing

Primer 2.5 μ l; 100% DMSO 3.5 μ l; amplification product 4.5 μ l; molecular grade water 6.0 μ l; 1/10 diluted Thermosequenase 3.0 μ l (TOTAL VOLUME 22 μ l). Five microliters of the foregoing master sequencing mix may then be added to each of the four tubes containing the deaza terminators. If necessary, the reaction mixtures may be covered with 8 μ l of mineral oil and subjected to the following cycle sequence:

- (1) denaturation at 94°C for 2 minutes, cycle 1X with
- (2) denaturation at 94°C for 30 seconds;
- (3) annealing at 55°C for 30 seconds, cycle 35X with
- (4) extension at 70°C for 60 seconds;
- (5) extension at 70°C for 2 minutes, cycle 1X; and
- (6) soak at 4°C.

The reaction products may then be run on a sequencing gel to ascertain the sequence of the amplification product, using standard techniques.

Methods of high resolution typing are detailed in the examples below, which examples are set out to exemplify the method of the invention and not to limit the scope of it in any way.

6. EXAMPLE: DETERMINATION OF HLA-A GROUP TYPE

Genomic DNA was prepared from patient samples according to standard methods, such as a standard salting-out procedure (as provided by the Puregene DNA Isolation Kit, Gentra Systems, Inc., Minneapolis) or by detergent and proteinase K treatment (Current Protocols in Molecular Biology, Eds. Ausubel, F.M. et al, (John Wiley & Sons; 1995)).

All primers were synthesized on a Gene Assembler plus (Pharmacia, Uppsala, Sweden), and purified by fast protein liquid chromatography. The sequence, length, melting temperature (T_m), group specificity localization of the primers are given in Tables 3 (sense primers), 4 (antisense primers) and 5 (primer pairs). Internal positive control primers were: 5' primer hGHI 5'GCC TTC CCA ACC ATT CCC TTA 3', (SEQ ID NO:336) 21mer, T_m =64°C, nucleotide position 5560-5580; 3' primer hGHI 5' TCC ATG TCC TTC CTG AAG CA 3', (SEQ ID NO:349) 20mer, T_m =60°C, nucleotide position 6614-6633. These control primers amplify a 1074 bp

fragment of the human growth hormone gene.

Group-specific identification was performed as follows. Aliquots of genomic DNA were separately reacted with a panel of 24 group-specific exon region primer pairs set forth in Table 5, *supra* (see Blasczyk et al., 1995, Tissue Ant. 46:86-95). An amplification cocktail for pairs of primers was prepared in 10 μ l volume using standard 10x Perkin-Elmer buffer (1x buffer: 50 mM KCl; 1.5 mM $MgCl_2$; 10 mM Tris-Hcl, pH 8.3; 0.001% (w/v) gelatin) supplemented with 5% glycerol and 0.1 μ l Cresol-red, sodium salt (Cresol-red stock solution: 10 mg/ml). The use of glycerol and cresol red avoids the necessity of using an agarose gel loading buffer. Additionally, glycerol increases the PCR yield.

The PCR mix for a single SSP tube was as follows:

Genomic DNA 100 ng	=	1.00 μ l
<i>Taq</i> polymerase, 0.4 U	=	0.08 μ l
dNTPs, 10 mM	=	0.80 μ l
Buffer, 10x	=	1.00 μ l
Glycerol	=	0.50 μ l
Cresol red 10mg/ml	=	0.10 μ l
dH ₂ O	=	1.52 μ l
Primer Pair + Control		
Primer Pair	=	5.00 μ l
Total		10.00 μ l

The PCR solution was prepared in volumes that would accommodate 30 reactions. The amount of primers used in each 10 μ l PCR volume was 3 pmol of each HJ A-A primer and 0.8 pmol of each internal control primer.

The reaction mixture was mixed well, then heated in a Thermo-Cycler 9600 (Perkin-Elmer, Inc) and subjected to the following protocol. After an initial denaturation, a first round with 10 two-temperature cycles was followed by 20 three-temperature cycles.

- 1) Initial denaturation at 95°C for 5 min.
- 2) First 10 cycles
 - i) Denaturation at 95°C for 30 sec.
 - ii) Annealing and extension at 65°C for 50 sec.
- 3) Last 20 cycles
 - i) Denaturation at 95°C for 30 sec.

ii) Annealing at 62°C for 50 sec.

iii) Extension at 72°C for 30 sec.

The reaction tube was then cooled on ice. For visualization, 8 ul of the amplification product were run on a 2 % agarose gel prestained with ethidium bromide (0.2 ug/ml). The results were compared to a control lane with known size markers. The reaction products were visualized either as two bands (alleles from different groups) or a single band (alleles from same group). The size of the band(s) were determined and group specificity was assigned according to the length assignments in Table 5.

Figures 10 and 11 show typical gel results, which, as shown in Tables 7 and 8, were interpreted to determine what group specificities were present in genomic DNA samples tested. In Tables 10 and 11, the column titled "Position" refers to the primer mix no. of Table 5.

Table 10.

<u>Position</u>	<u>HLA Specificity</u>	<u>Kontr. Species ampl.</u>	<u>PM</u>
1	A*0101,0102,8001		1
2	A*0201-0217		2
3	A*0301,0302		3
4	A*0101,3601		36
5	A*1101,1102		11
6	A*2301		23
7	A*2402-2407		24
8	A*2603,2605,6601	X	10.1
9	A*2501		25
10	A*2601,2602,2604,4301		26
11	A*3401,3402		34
12	A*6602		6602
13	A*1101,1102,3401,3402, 6601,6602, A*68011,6802,6901	X	10.2
14	A*4301		43
15	A*68012,6802,6803		68
16	A*6901		69
17	A*2901,2902,3101,3201 3301-3303, A*7401	X	19
18	A*2901,2902		29
19	A*3001-3004		30
20	A*3101		31
21	A*3201,2501	X	32

Table 11.

Position	HLA Specificity	Kontr. Species Ampl.	PM
1	A*0101,0102,8001		
2	A*0201-0217		
3	A*0301,0302	X	1
4	A*0101,3601		2
5	A*1101,1102		3
6	A*2301		36
7	A*2402-2407		11
8	A*2501,2601-2603, 2605,6601		23 24
9	A*2501		10.1
10	A*2601,2602,2604,4301		25
11	A*3401,3402		26
12	A*6602		34
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14	A*4301		
15	A*6801,6802		
16	A*6901	X	43
17	A*2901-2902,3101,3201, 3301-3303, A*7401		68 69
18	A*2901,2902		19
19	A*3001-3004		29 30

7. EXAMPLE: DETERMINATION OF GROUP SPECIFICITY USING A PRIMER COCKTAIL

Group specific low-resolution typing of the patient sample may be performed as follows. First, a stock PCR amplification reaction mixture may be prepared for 30 reactions:

dNTPs 10mM	<u>41</u>
Glycerol 100%	24
10x PCR Buffer*	15
Cresol-red (10mg/ml)	30
H2O	3.0
final	<u>45</u>
	117

*1 X PCR Buffer comprises 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl₂ and 0.001% (w/v) gelatin.

The stock mixture may be prepared in a large volume and be stored for at least one month at 4°C or be aliquoted (117.0 µl) and stored at -30°C for at least six months. Repeated thawing and freezing should be avoided.

A mixture containing all the HLA-A group specific amplification primers listed in Table 5 may be prepared separately (the "Cocktail"). One member of each primer pair is labelled on the 5' end with a fluorescent label. Final Cocktail concentrations may be designed to provide 3 pmol of each HLA-A primer per 5 µl. Optionally, an internal control primer may be added (to determine among other things, the success of amplification) in the amount of 0.8 pmol per 5 µl. Suitable internal control primers amplify a 1074 bp fragment of the human growth hormone gene (*see supra*).

To perform the low resolution amplification reaction, the reaction mixture may be prepared as follows:

	Volume
Stock Mixture	5 µl
Cocktail	5 µl
Patient sample DNA 100-250 ng	1 µl
Taq Polymerase Enzyme 0.4 U	0.08 µl

PCR cycle parameters may be adjusted for a Perkin-Elmer System 9600 thermal cycler. After an initial denaturation, a first round with 10 two-temperature cycles may be followed by 20 three-temperature cycles: 1) Initial Denaturation at 95°C for 5 min; 2) First 10 cycles i) Denaturation at 95°C for 30 seconds and ii) Annealing and extension at 65°C for 50 seconds; 3) Last 20 cycles i) Denaturation at 95°C for 30 seconds, ii) Annealing at 62°C for 50 seconds and iii) Extension at 72°C for 30 seconds.

The reaction tube may then be cooled on ice. For visualization, 2 µl of the amplification product may be run on a polyacrylamide gel giving single nucleotide length resolution such as in a MicroGene Blaster. The results were compared to a control lane with known size markers. The reaction products may be visualized either

as two bands (alleles from different groups) or a single band (alleles from same group). The size of the band(s) may be determined and group specificity may be assigned according to the length assignments in Table 5.

8. EXAMPLE: DETERMINATION OF ALLELIC TYPE BY SEQUENCING

After determining group type specificity, group specific amplification of a fresh portion of the patient sample may be performed using a single pair of primers specific for the group in question to generate sequencing template. In a preferred, nonlimiting embodiment, amplification primers may be selected from Table 2, *supra*, which lists group-specific untranslated region primers. This second amplification serves two purposes. First, it confirms, by successful amplification, the group determination of the low resolution test. Second, it generates sequence information which may be used for accurate allele identification. If two groups are identified, two separate reactions may be performed each using a different primer pair.

8.1. PCR PROTOCOL

The same PCR protocol may be used for all primer mixes used for template generation. The PCR amplification may be set up in a total volume of 50 μ l in order to produce enough PCR product for more than 10 sequencing reactions. This ensures that, if anything fails during the sequencing process, sequencing can be repeated without generation of a new template. The high stringency of the PCR primers and protocol detailed below makes the use of a "hot start approach" unnecessary. The following PCR reaction mix may be used:

<u>volume per reaction</u>	
5X PCR buffer*	10.0 μ l
DMSO	1.0 μ l
2.5mM each dNTP	5.0 μ l
ddH ₂ O	<u>27.8μl</u>
Total	43.8 μ l
Sense primer** (10pmol/ μ l)	1.0 μ l
Antisense primer** (10pmol/ μ l)	1.0 μ l
Taq Polymerase (5U/ μ l)	0.2 μ l
Genomic DNA (100ng/ μ l)	<u>4.0μl</u>
Final Total	50.0 μ l

*Composition of 5X PCR buffer: 75mM (NH₄)₂SO₄; 17.5mM MgCl₂; and 300mM Tris-HCL, pH 9.0

**The pair of group specific amplification primers may be selected from those disclosed in Table 2, *supra*.

PCR cycle parameters may be adjusted for a Perkin-Elmer System 9600 thermal cycler. After an initial denaturation, a first round with 10 two-temperature cycles may be followed by 20 three-temperature cycles.

- 1.) Initial Denaturation at 95 C for 5 min
- 2.) First 10 cycles
 - i) Denaturation at 95 C for 30 seconds
 - ii) Annealing and extension at 65 C for 50 seconds
- 3.) Last 20 cycles
 - i) Denaturation at 95 C for 30 seconds
 - ii) Annealing at 62 C for 50 seconds
 - iii) Extension at 72 C for 30 seconds

10 μ l of the PCR product may then be run on a 2 % agarose gel prestained with ethidium bromide (0,2 μ g/ml). A distinct band of the expected size should be seen.

8.2. SEQUENCING REACTION PROTOCOL

The sequencing reactions may be carried out with AmpliTaqTM DNA Polymerase FS dye terminator cycle sequencing chemistry using the Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit FS (Perkin Elmer Applied Biosystems Division, Foster City, CA) according to the manufacturer's protocol. This Kit contains the four ddNTPs with different fluorescence labels (=Dye Terminators). The PCR fragments may be used directly for sequencing without any prior purification step.

To simplify the pipetting steps, a master mix may be prepared consisting of the 5'Biotin labeled sequencing primer, ddH₂O and the Kit reagents. This master mix should be prepared immediately prior to use and can be kept at room temperature until use. The sequencing master mix for one reaction may comprise 3.0 μ l of a 1pmol/ μ l solution of sequencing primer; 6.0 μ l ddH₂O, and 8.0 μ l of premixed sequencing reagents; for 36 + 1 reactions, these amounts are increased, respectively,

to 111.0 μ l; 222.0 μ l; and 296.0 μ l, respectively. The sequencing primer may be selected from the sequencing primers for HLA-A set forth in Table 6, *supra*.

The master mix may be aliquoted in a volume of 17 μ l for each sequencing reaction in a 200 μ l PCR tube and 3 μ l of the unpurified PCR product are added. The reaction mixes may then be subjected to 25 cycles in a Perkin Elmer thermal cycler 9600. Each cycle consists of 10 sec 95 C, 5 sec 50 C and 4 min 60 C.

8.3. PURIFICATION OF EXTENSION PRODUCTS

After the sequencing reaction the extension products are desirably separated from the unincorporated Dye Terminators which would otherwise interfere with the fluorescence-based detection process of the electrophoretically separated sequencing fragments.

For each sequencing reaction, 50 μ g (5 μ l) Streptavidin-coated Dynabeads M-280 (Dynal Inc., Oslo, Norway) may be washed in 5 μ l of 2x Binding and Washing buffer ("B&W"; 2X B&W buffer: 2M NaCl, 10mM Tris-HCl pH 7.5, 1mM EDTA). The beads may then be resuspended in 20 μ l of 2x B&W.

To each 20 μ l sequencing reaction, 20 μ l of resuspended beads may be added, and the mixture may be incubated at room temperature (20-25 C) for 15 minutes. The beads may then be immobilized, the supernatant may be removed, and then the beads may be washed once in 70% ethanol by pipetting up and down five times. Then, as much as possible of the ethanol may desirably be removed, because residual ethanol may interfere with electrophoretic gel mobility.

For each sequencing reaction, 4 μ l of loading buffer (5:1 Formamide-25mM EDTA pH 8.0, 50mg/ml Dextran Blue) may be added.

8.4. ELECTROPHORESIS AND DATA COLLECTION

Samples prepared by the foregoing methods may be used immediately or be stored at 4 C at least for 24 hours before starting the electrophoretic separation. Prior to the electrophoretic separation, each reaction may be incubated at 90 C for 2 minutes. 3 μ l of each sample may be loaded on a prerun sequencing gel. For an automated ABI 377 sequencer (Applied Biosystems, Foster City, CA) a 0.2mm thick 5% polyacrylamide (acrylamide:bisacrylamide = 29:1) - 7 M urea gel may be used

[gel composition: 21.0 g urea, 8.4 ml 30% acrylamide (stock solution: 58g acrylamide, 2g bisacrylamide in bidistilled water), 6.0 ml TBE buffer (10x TBE-buffer: 108.0 g tris base, 55.0 g boric acid, 7.4 g Na₂EDTA), 15 μ l TEMED, 350 μ l 10% Ammoniumpersulfate (1.0 g Ammoniumpersulfate in 10 ml ddH₂O), 20.0 ml ddH₂O]. Electrophoresis may be run at constant 48 watt for 8h. Data collection may be initiated immediately after starting the electrophoresis on the ABI377. Data analyses may be performed thereafter using the ABI analysis software (version 2.1.1).

8.5. DATA INTERPRETATION AND HLA TYPING

After data collection, the chromatograms may be printed and sequences may be compared manually to existing HLA data in the EMBL databank and the sequences compiled by Arnett and Parham. Due to the group-specific amplification and the lack of heterozygous positions, manual analysis is typically very fast. Alternatively, sequences may be checked with the data analysis editor (Sequence NavigatorTM, Applied Biosystems) and aligned with any sequence alignment program.

Various publications are cited herein, the contents of which are hereby incorporated by reference in their entireties.

SEQUENCE LISTING

<110> BLASCZYK, RAINER
LEUSHNER, JAMES

<120> METHOD AND KIT FOR CLASS I HLA TYPING

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cggttcgcga gacagagtta cagagggact tagaaccggg ttctcgacag actctttgtt 360
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<400> 20

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cagggacttg acctgaggga ctgagggtg 449
  
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<210> 21
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<212> DNA

<213> homo sapiens

<400> 21

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cggttcgaga gacagagta cagagggact cagaaccggg ttctcgacag actctttgtt   360
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<211> 449

<212> DNA

<213> homo sapiens

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acgcctcaac ccttagggg ttccgacct gaggggtag gtatgtggcg gaagccccgg   240
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cggttcgaga gacagagta cagagggact cagaaccggg ttctcgacag actctttgtt   360
ccctctttgg gagccgtacc cggggcaggg agaggaaagt gaaaaatagg gcattagaga   420
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<210> 23

<211> 449

<212> DNA

<213> homo sapiens

<400> 23

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gcgagcact cataggtcct tcttctggg atgtatcaa ccctctcct cttttcttg   180
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cggttcgaga gacagagta cagagggact cagaaccggg ttctcgacag actctttgtt   360
ccctctttgg gagccgtacc cggggcaggg agaggaaagt gaaaaatagg gcattagaga   420
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<210> 24

<211> 449

<212> DNA

<213> homo sapiens

<400> 24

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<210> 25

<211> 449

<212> DNA

<213> homo sapiens

<400> 25

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 gcgcagcact cataggtcct tcttctggg atgtatccaa cctctccct cttttcttg 180
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 cggttcgga gacagagta cagagggact cagaaccggg ttctcgacag actctttgt 360
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<210> 26

<211> 449

<212> DNA

<213> homo sapiens

<400> 26

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 gcgcagcact cataggtcct tcttctggg atgtatccaa cctctccct cttttcttg 180
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<210> 27

<211> 449

<212> DNA

<213> homo sapiens

<400> 27

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 cggttcgga gacagagtta cagagggact cagaaccggg ttctcgacag actctttgta 360
 cctcttttg gagccgtacc cggggcaggg agaggaaagt gaaaatagg gcattagaga 420
 cagggacttg acctgaggga ctgagggtg 449

<210> 28

<211> 449

<212> DNA

<213> homo sapiens

<400> 28

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 gcgcagcact cataggtcct tcttctggg atgtatccaa cctctccct cttttctttg 180
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 cggttcgaga gacagagtta cagagggact cagaaccggg ttctcgacag actctttgtt 360
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<210> 29

<211> 449

<212> DNA

<213> homo sapiens

<400> 29

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 cggttcgaga gacagagtta cagagggact cagaaccggg ttctcgacag actctttgtt 360
 cctcttttg gagccgtacc cggggcaggg agaggaaagt gaaaagtagg gcattagaga 420
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<210> 30

<211> 449

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<213> homo sapiens

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 gcgcagcact cataggtcct tcttctggg atgtatcaa cctctccct cttttcttg 180
 acgcctcaac cccttagggg ttccgacct gaggggtag gtatgtggcg gaagccccgg 240
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 cggttcgga gacagagta cagagggact cagaaccggg ttctcgacag actctttgtt 360
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 cagggacttg acctgaggga ctgagggtg 449

<210> 31
 <211> 449
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 <213> homo sapiens

<400> 31
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 gcgcagcact cataggtcct tcttctggg atgtatcaa cctctccct cttttcttg 180
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 cggttcgga gacagagta cagagggact cagaaccggg ttctcgacag actctttgtt 360
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<210> 32
 <211> 449
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<400> 32
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 gcgcagcact cataggtcct tcttctggg atgtatcaa cctctccct cttttcttg 180
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 cggttcgga gacagagta cagagggact cagaaccggg ttctcgacag actctttgtt 360
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<210> 33
 <211> 449
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<400> 33
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 cggttcgca gacagagta cagagggact cagaaccggg ttctcgacag actctttgtt 360
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<210> 34
 <211> 449
 <212> DNA
 <213> homo sapiens

<400> 34
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 cggttcgca gacagagta cagagggact cagaaccggg ttctcgacag actctttgtt 360
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 cagggacttg acctgaggga ctgagggtg 449

<210> 35
 <211> 18
 <212> DNA
 <213> homo sapiens

<400> 35
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<210> 36
 <211> 449
 <212> DNA
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<400> 36
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 gcgcagcact cataggtcct tcttctggg atgtatccaa cctctccct cttttcttg 180
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<210> 37
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<210> 38
 <211> 449
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 <213> homo sapiens

<400> 38
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 gcgcagcact cataggtcct tcttctggg atgtatccaa ccctctccct ctttctttg 180
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 cggttcgcga gacagagta cagagggact cagaaccggg tctcgacag actctttgtt 360
 cctcttttg gagccgtacc cggggcaggg agaggaaagt gaaaagtagg gcattagaga 420
 cagggacttg acctgaggga ctgagggtg 449

<210> 39
 <211> 130
 <212> DNA
 <213> homo sapiens

<400> 39
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 gggggcgag gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
 tcgccccag 130

<210> 40
 <211> 130
 <212> DNA
 <213> homo sapiens

<400> 40

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 gggggcgcag gaccggggga gccgcgccgg gaggaggggc gggcaggtct cagccactgc 120
 tcgccccag 130

<210> 41
 <211> 130
 <212> DNA
 <213> homo sapiens

<400> 41
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 gggggcgcag gaccggggga gccgcgccgg gaggaggggc gggcaggtct cagccactgc 120
 tcgccccag 130

<210> 42
 <211> 130
 <212> DNA
 <213> homo sapiens

<400> 42
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 tcgccccag 130

<210> 43
 <211> 130
 <212> DNA
 <213> homo sapiens

<400> 43
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 gggggcgcag gaccggggga gccgcgccgg gaggaggggc gggcaggtct cagccactgc 120
 tcgccccag 130

<210> 44
 <211> 130
 <212> DNA
 <213> homo sapiens

<400> 44
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 gggggcgcag gaccggggga gccgcgccgg gaggaggggc ggtcaggtct cagccactgc 120
 tcgccccag 130

<210> 45
 <211> 130

<212> DNA

<213> homo sapiens

<400> 45

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ggggggcgag gaccggggga gccgcgccgg gaggagggtc ggtcagggtc cagccactgc 120
tcgccccag                                     130
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<210> 46

<211> 130

<212> DNA

<213> homo sapiens

<400> 46

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ggggggcgag gaccggggga gccgcgccgg gaggagggtc ggtcagggtc cagccactgc 120
tcgccccag                                     130
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<210> 47

<211> 130

<212> DNA

<213> homo sapiens

<400> 47

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ggggggcgag gaccggggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtccccag                                     130
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<210> 48

<211> 130

<212> DNA

<213> homo sapiens

<400> 48

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ggggggcgag gaccggggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtccccag                                     130
```

<210> 49

<211> 130

<212> DNA

<213> homo sapiens

<400> 49

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ggggggcgag gaccggggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
```

<210> 50
<211> 130
<212> DNA
<213> homo sapiens

<400> 50
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ggggggcgag gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtccccag 130

<210> 51
<211> 130
<212> DNA
<213> homo sapiens

<400> 51
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ggggggcgag gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtccccag 130

<210> 52
<211> 130
<212> DNA
<213> homo sapiens

<400> 52
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ggggggcgag gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtccccag 130

<210> 53
<211> 130
<212> DNA
<213> homo sapiens

<400> 53
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ggggggcgag gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtccccag 130

<210> 54
<211> 130
<212> DNA
<213> homo sapiens

<400> 54

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gggggcgcag gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtcccag 130

<210> 55

<211> 130

<212> DNA

<213> homo sapiens

<400> 55

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gggggcgcag gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtcccag 130

<210> 56

<211> 130

<212> DNA

<213> homo sapiens

<400> 56

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gggggcgcag gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtcccag 130

<210> 57

<211> 130

<212> DNA

<213> homo sapiens

<400> 57

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gggggcgcag gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtcccag 130

<210> 58

<211> 130

<212> DNA

<213> homo sapiens

<400> 58

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gggggcgcag gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtcccag 130

<210> 59

<211> 130
 <212> DNA
 <213> homo sapiens

<400> 59

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ggggg'gcag gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtcccag                                     130
```

<210> 60
 <211> 130
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 <213> homo sapiens

<400> 60

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ggggg'gcag gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtcccag                                     130
```

<210> 61
 <211> 130
 <212> DNA
 <213> homo sapiens

<400> 61

```
gtgagtgcgg ggtcgggagg gaaacggcct ctgtggggag aagcaagggg cccgcccggc 60
ggggg'gcag gacccgggaa gccgcgccgt gaggagggtc gggcgggtct cagccactcc 120
tcgccccag                                     130
```

<210> 62
 <211> 130
 <212> DNA
 <213> homo sapiens

<400> 62

```
gtgagtgcgg ggtcgggagg gaaacggcct ctgtggggag aagcaagg gg cccgcccggc 60
ggggg'gcag gacccgggaa gccgcgccgt gaggagggtc gggcgggtct cagccactcc 120
tcgccccag                                     130
```

<210> 63
 <211> 130
 <212> DNA
 <213> homo sapiens

<400> 63

```
gtgagtgcgg ggtcgggagg gaaacggcct ctgtggggag aagcaagggg cccgcccggc 60
```

gggggagcag gacccgggaa gccgcgcctg gaggagggtc gggcgggtct cagccactcc 120
tcgtcccag 130

<210> 64
<211> 130
<212> DNA
<213> homo sapiens

<400> 64
gtgagtgcgg ggtcgggagg gaaacggcct ctgcggggag aagcaagggg ccgcctggc 60
gggggagcaa gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtcccag 130

<210> 65
<211> 130
<212> DNA
<213> homo sapiens

<400> 65
gtgagtgcgg ggtcgggagg gaaacggcct ctgcggggag aagcaagggg ccgcctggc 60
gggggagcaa gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtcccag 130

<210> 66
<211> 130
<212> DNA
<213> homo sapiens

<400> 66
gtgagtgcgg ggtcgggagg gaaacggcct ctgcggggag aagcaagggg ccgcctggc 60
gggggagcaa gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtcccag 130

<210> 67
<211> 130
<212> DNA
<213> homo sapiens

<400> 67
gtgagtgcgg ggtcgggagg gaaacggcct ctgcggggag aagcaagggg ccgcctggc 60
gggggagcaa gacccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc 120
tcgtcccag 130

<210> 68
<211> 130
<212> DNA

<213> homo sapiens

<400> 68

```

gtgagtgcgg ggtcgggagg gaaacggcct ctgcggggag aagcaagggg cccgcctggc   60
gggggcgcaa gaccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc   120
tcgtcccag                                     130

```

<210> 69

<211> 130

<212> DNA

<213> homo sapiens

<400> 69

```

gtgagtgcgg ggtcgggagg gaaacggcct ctgcggggag aagcaagggg cccgcctggc   60
gggggcgcaa gaccgggaa gccgcgccgg gaggagggtc gggcgggtct cagccactcc   120
tcgtcccag                                     130

```

<210> 70

<211> 129

<212> DNA

<213> homo sapiens

<400> 70

```

gtgagtgcgg ggtcgtgggg aaaccgcctc tgcggggaga agcaaggggc ccgcccggcg   60
gggacgcagg acccggttag ccgcgccggg aggagggtcg ggtgggtctc agccactct   120
cgccccag                                     129

```

<210> 71

<211> 130

<212> DNA

<213> homo sapiens

<400> 71

```

gtgagtgcgg ggtcgggagg gaaacggcct ctgtggggag aagcaagggg cccgcccggc   60
gggggcgcag gaccgggaa gccgcgcctg gaggagggtc gggcgggtct cagccactcc   120
tcgccccag                                     130

```

<210> 72

<211> 130

<212> DNA

<213> homo sapiens

<400> 72

```

gtgagtgcgg ggtcgggagg gaaacggcct ctgtggggag aagcaagggg cccgcccggc   60
gggggcgcag gaccgggaa gccgcgcctg gaggagggtc gggcgggtct cagccactcc   120
tcgccccag                                     130

```

<210> 73
 <211> 130
 <212> DNA
 <213> homo sapiens

<400> 73
 gtgagtgcgg ggtcgggagg gaaacggcct ctgtggggag aagcaagggg cccgcccggc 60
 gggggcgcag gacccgggaa gccgcgcctg gaggagggtc gggcgggtct cagccactcc 120
 tcgccccag 130

<210> 74
 <211> 130
 <212> DNA
 <213> homo sapiens

<400> 74
 gtgagtgcgg ggtcgggagg gaaacggcct ctgtggggag aagcaagggg cccgcccggc 60
 gggggcgcag gacccgggaa gccgcgcctg gaggagggtc gggcgggtct cagccactcc 120
 tcgccccag 130

<210> 75
 <211> 130
 <212> DNA
 <213> homo sapiens

<400> 75
 gtgagtgcgg ggtcgggagg gaaacggcct ctgtggggag aagcaagggg cccgcccggc 60
 gggggcgcag gacccgggaa gccgcgcctg gaggagggtc gggcgggtct cagccactcc 120
 tcgccccag 130

<210> 76
 <211> 130
 <212> DNA
 <213> homo sapiens

<400> 76
 gtgagtgcgg ggtcgggagg gaaacggcct ctgtggggag aagcaagggg cccgcccggc 60
 gggggcgcag gacccgggaa gccgcgcctg gaggagggtc gggcgggtct cagccactcc 120
 tcgccccag 130

<210> 77
 <211> 129
 <212> DNA
 <213> homo sapiens

<400> 77

gtgagtgcgg ggctgtgggg aaaccgcctc tgcggggaga agcaaggggc cgcgccggcg 60
 gggacgcagg acccgggtag ccgcgccggg aggagggtcg ggtgggtctc agccactcct 120
 cgccccag 129

<210> 78

<211> 129

<212> DNA

<213> homo sapiens

<400> 78

gtgagtgcgg ggctgtgggg aaaccgcctc tgcggggaga agcaaggggc cgcgccggcg 60
 gggacgcagg acccgggtag ccgcgccggg aggagggtcg ggtgggtctc agccactcct 120
 cgccccag 129

<210> 79

<211> 129

<212> DNA

<213> homo sapiens

<400> 79

gtgagtgcgg ggctgtgggg aaaccgcctc tgcggggaga agcaaggggc cgcgccggcg 60
 ggggcgccagg acccgggtag ccgcgccggg aggagggtcg ggcgggtctc agccactcct 120
 cgccccag 129

<210> 80

<211> 129

<212> DNA

<213> homo sapiens

<400> 80

gtgagtgcgg ggctgtgggg aaaccgcctc tgcggggaga agcaaggggc cgcgccggcg 60
 ggggcgccagg acccgggtag ccgcgccggg aggagggtcg ggcgggtctc agccactcct 120
 cgccccag 129

<210> 81

<211> 129

<212> DNA

<213> homo sapiens

<400> 81

gtgagtgcgg ggctgtgggg aaaccgcctc tgcggggaga agcaaggggc cgcgccggcg 60
 ggggcgccagg acccgggtag ccgcgccggg aggagggtcg ggcgggtctc agccactcct 120
 cgccccag 129

<210> 82

<211> 129

<212> DNA

<213> homo sapiens

<400> 82

```

gtgagtgcgg ggtcgtgggg aaaccgcctc tgcggggaga agcaaggggc ccgcccggcg   60
ggggcgcagg acccgggtag ccgcgccggg aggagggtcg ggcggatctc agccactct   120
cgccccag                                     129

```

<210> 83

<211> 129

<212> DNA

<213> homo sapiens

<400> 83

```

gtgagtgcgg ggtcgtgggg aaaccgcctc tgcggggaga agcaaggggc ccgcccggcg   60
ggggcgcagg acccgggtag ccgcgccggg aggagggtcg ggcgggtctc agccactct   120
cgccccag                                     129

```

<210> 84

<211> 129

<212> DNA

<213> homo sapiens

<400> 84

```

gtgagtgcgg ggtcgtgggg aaaccgcctc tgcggggaga agcaaggggc ccgcccggcg   60
ggggcgcagg acccgggtag ccgcgccggg aggagggtcg ggcgggtctc agccactct   120
cgccccag                                     129

```

<210> 85

<211> 129

<212> DNA

<213> homo sapiens

<400> 85

```

gtgagtgcgg ggtcgtgggg aaaccgcctc tgcggggaga agcaaggggc tcgcccggcg   60
ggggcgcagg acccgggtag ccgcgccggg aggagggtcg ggcgggtctc agccactct   120
cgccccag                                     129

```

<210> 86

<211> 130

<212> DNA

<213> homo sapiens

<400> 86

```

gtgagtgcgg ggtcgggagg gaaacggcct ctgcggggag aagcaagggg cccgcccggc   60
ggggcgcag gacccgggaa gccgcgccgg gaggagggtc ggcgggtct cagccactcc   120

```

tcgccccag

130

<210> 87

<211> 241

<212> DNA

<213> homo sapiens

<400> 87

gtgagtacc ccggcccggg gcgcaggta cgacccctca tccccacgg acgggccagg 60
tcgcccacag tctccgggtc cgagatccac cccgaagccg cgggaccccg agacccttgc 120
ccggggagag gccagggcgc ctttaccggg ttctatttc agtttaggcc aaaaatcccc 180
ccgggttggg cggggcgggg cggggctcgg gggactgggc tgaccgcggg gtcggggcca 240
g 241

<210> 88

<211> 241

<212> DNA

<213> homo sapiens

<400> 88

gtgagtacc ccggcccggg gcgcaggta cgacccctca tccccacgg acgggccagg 60
tcgcccacag tctccgggtc cgagatccac cccgaagccg cgggactccg agacccttgc 120
ccggggagag gccagggcgc ctttaccggg ttctatttc agtttaggcc aaaaatcccc 180
ccgggttggg cggggcgggg cggggctcgg gggactgggc tgaccgcggg gtcggggcca 240
g 241

<210> 89

<211> 241

<212> DNA

<213> homo sapiens

<400> 89

gtgagtacc ccggcccggg gcgcaggta cgacctctca tccccacgg acgggccagg 60
tcgcccacag tctccgggtc cgagatccgc cccgaagccg cgggaccccg agacccttgc 120
ccggggagag gccagggcgc ctttaccggg ttctatttc agtttaggcc aaaaatcccc 180
ccaggttggg cggggcgggg cggggctcgg gggaccgggc tgaccgcggg gtcggggcca 240
g 241

<210> 90

<211> 241

<212> DNA

<213> homo sapiens

<400> 90

gtgagtacc ccggcccggg gcgcaggta cgacctctca tccccacgg acgggccagg 60
tcgcccacag tctccgggtc cgagatccgc cccgaagccg cgggaccccg agacccttgc 120

cccgaggagag gccagggcgc cttaccggg ttccatttc agtttaggcc aaaaatcccc 180
ccaggttggg cggggcgggg cggggctcgg gggaccgggc tgaccgcggg gtccgggcca 240
g 241

<210> 91
<211> 241
<212> DNA
<213> homo sapiens

<400> 91
gtgagtacc ccggcccggg gcgcaggta cgacctctca tccccacgg acgggccagg 60
tcgccacag tctccgggtc cgagatccgc ccgaagccg cgggacccc agacccttc 120
cccgaggagag gccagggcgc cttaccggg ttccatttc agtttaggcc aaaaatcccc 180
ccaggttggg cggggcgggg cggggctcgg gggaccgggc tgaccgcggg gtccgggcca 240
g 241

<210> 92
<211> 241
<212> DNA
<213> homo sapiens

<400> 92
gtgagtacc ccggcccggg gcgcaggta cgacctctca tccccacgg acgggccagg 60
tcgccacag tctccgggtc cgagatccgc ccgaagccg cgggacccc agacccttc 120
cccgaggagag gccagggcgc cttaccggg ttccatttc agtttaggcc aaaaatcccc 180
ccaggttggg cggggcgggg cggggctcgg gggaccgggc tgaccgcggg gtccgggcca 240
g 241

<210> 93
<211> 241
<212> DNA
<213> homo sapiens

<400> 93
gtgagtacc ccggcccggg gcgcaggta cgacctctca tccccacgg acgggccagg 60
tcgccacag tctccgggtc cgagatccgc ccgaagccg cgggacccc agacccttc 120
cccgaggagag gccagggcgc cttaccggg ttccatttc agtttaggcc aaaaatcccc 180
ccaggttggg cggggcgggg cggggctcgg gggaccgggc tgaccgcggg gtccgggcca 240
g 241

<210> 94
<211> 241
<212> DNA
<213> homo sapiens

<400> 94

```

gtgagtgacc ccggcccggg ggcaggtca cgacctctca tccccacgg acgggccagg 60
tcgcccacag tctccgggtc cgagatccgc cccgaagccg cgggacccc agacccttgc 120
cccgggagag gccagggcgc cttaccggg ttctatttc agtttaggcc aaaaatcccc 180
ccaggttggt cggggcgggg cggggctcgg gggaccgggc tgaccgcggg gtccggggcca 240
g
241

```

<210> 95
 <211> 241
 <212> DNA
 <213> homo sapiens

```

<400> 95
gtgagtgacc ccggcccggg ggcaggtca cgacctctca tccccacgg acgggccagg 60
tcgcccacag tctccgggtc cgagatccgc cccgaagccg cgggacccc agacccttgc 120
cccgggagag gccagggcgc cttaccggg ttctatttc agtttaggcc aaaaatcccc 180
ccaggttggt cggggcgggg cggggctcgg gggaccgggc tgaccgcggg gtccggggcca 240
g
241

```

<210> 96
 <211> 241
 <212> DNA
 <213> homo sapiens

```

<400> 96
gtgagtgacc ccggcccggg ggcaggtca cgacctctca tccccacgg acgggccagg 60
tcgcccacag tctccgggtc cgagatccgc cccgaagccg cgggacccc agacccttgc 120
cccgggagag gccagggcgc cttaccggg ttctatttc agtttaggcc aaaaatcccc 180
ccaggttggt cggggcgggg cggggctcgg gggaccgggc tgaccgcggg gtccggggcca 240
g
241

```

<210> 97
 <211> 241
 <212> DNA
 <213> homo sapiens

```

<400> 97
gtgagtgacc ccggcccggg ggcaggtca cgacctctca tccccacgg acgggccagg 60
tcgcccacag tctccgggtc cgagatccgc cccgaagccg cgggacccc agacccttgc 120
cccgggagag gccagggcgc cttaccggg ttctatttc agtttaggcc aaaaatcccc 180
ccaggttggt cggggcgggg cggggctcgg gggaccgggc tgaccgcggg gtccggggcca 240
g
241

```

<210> 98
 <211> 241
 <212> DNA
 <213> homo sapiens

<400> 98

```

gtgagtgacc ccggcccggg gcgcaggta cgaactctca tccccacgg acgggccagg   60
tcgccacag tctccgggtc cgagatccgc ccgaagccg cgggacccc agaccctgc   120
ccgggagag gccaggcgc cttaccggg ttcatcttc agtttaggc aaaaatccc   180
ccaggttgt cggggcgggg cggggctcg gggaccggg tgaccgggg gtccgggcca   240
g

```

<210> 99

<211> 241

<212> DNA

<213> homo sapiens

<400> 99

```

gtgagtgacc ccggcccggg gcgcaggta cgaactctca tccccacgg acgggccagg   60
tcgccacag tctccgggtc cgagatccgc ccgaagccg cgggacccc agaccctgc   120
ccgggagag gccaggcgc cttaccggg ttcatcttc agtttaggc aaaaatccc   180
ccaggttgt cggggcgggg cggggctcg gggaccggg tgaccgggg gtccgggcca   240
g

```

<210> 100

<211> 241

<212> DNA

<213> homo sapiens

<400> 100

```

gtgagtgacc ccggcccggg gcgcaggta cgaactctca tccccacgg acgggccagg   60
tcgccacag tctccgggtc cgagatccgc ccgaagccg cgggacccc agaccctgc   120
ccgggagag gccaggcgc cttaccggg ttcatcttc agtttaggc aaaaatccc   180
ccaggttgt cggggcgggg cggggctcg gggaccggg tgaccgggg gtccgggcca   240
g

```

<210> 101

<211> 241

<212> DNA

<213> homo sapiens

<400> 101

```

gtgagtgacc ccggcccggg gcgcaggta cgaactctca tccccacgg acgggccagg   60
tcgccacag tctccgggtc cgagatccgc ccgaagccg cgggacccc agaccctgc   120
ccgggagag gccaggcgc cttaccggg ttcatcttc agtttaggc aaaaatccc   180
ccaggttgt cggggcgggg cggggctcg gggaccggg tgaccgggg gtccgggcca   240
g

```

<210> 102

<211> 241

<212> DNA

<213> homo sapiens

<400> 102

```
gtgagtgacc ccggcccggg ggcaggtca cgacccctca tccccacgg acgggccagg 60
tcgccacag tctccgggtc cgagatccgc cccgaagccg cgggacccc agacccttgc 120
cccgggagag gccagggcg ctttaccgg ttcatcttc agtttaggc aaaaatcccc 180
ccgggttggc cggggcgggg cggggctcgg gggaccgggc tgacctcggg gtccggggcca 240
g 241
```

<210> 103

<211> 241

<212> DNA

<213> homo sapiens

<400> 103

```
gtgagtgacc ccggcccggg ggcaggtca cgacccctca tccccacgg acgggccagg 60
tcgccacag tctccgggtc cgagatccgc cccgaagccg cgggacccc agacccttgc 120
cccgggagag gccagggcg ctttaccgg ttcatcttc agtttaggc aaaaatcccc 180
ccgggttggc cggggcgggg cggggctcgg gggaccgggc tgacctcggg gtccggggcca 240
g 241
```

<210> 104

<211> 241

<212> DNA

<213> homo sapiens

<400> 104

```
gtgagtgacc ccggcccggg ggcaggtca cgacctctca tccccacgg acgggccagg 60
tcgccacag tctccgggtc cgagatccgc cccgaagccg cgggacccc agacccttgc 120
cccgggagag gccagggcg ctttaccgg ttcatcttc agtttaggc aaaaatcccc 180
ccaggttggc cggggcgggg cggggctcgg gggaccgggc tgacctcggg gtccggggcca 240
g 241
```

<210> 105

<211> 241

<212> DNA

<213> homo sapiens

<400> 105

```
gtgagtgacc ccggcccggg ggcaggtca cgacccctca tccccacgg acgggccagg 60
tcgccacag tctccgggtc cgagatccgc cccgaagccg cgggacccc agacccttgc 120
cccgggagag gccagggcg ctttaccgg ttcatcttc agtttaggc aaaaatcccc 180
ccgggttggc cggggcgggg cggggctcgg gggaccgggc tgacctcggg gtccggggcca 240
g 241
```

<210> 106

<211> 241
 <212> DNA
 <213> homo sapiens

<400> 106

```
gtgagtgacc cgggcccggg gcgcaggta cgaacctca tccccacgg acgggccagg 60
tcgccacag tctccgggtc cgagatccgc cccgaagccg cgggaccccg agacccttgc 120
cccgggagag gccagggcgc cttaccggg ttcatcttc agtttaggcc aaaaatcccc 180
ccgggttggt cggggcgggg cggggctcgg gggaccgggc tgacctcggg gtccgggcca 240
g                                     241
```

<210> 107
 <211> 241
 <212> DNA
 <213> homo sapiens

<400> 107

```
gtgagtgacc cgggcccggg gcgcaggta cgaacctca tccccacgg acgggccagg 60
tcgccacag tctccgggtc cgagatccgc cccgaagccg cgggaccccg agacccttgc 120
cccgggagag gccagggcgc cttaccggg ttcatcttc agtttaggcc aaaaatcccc 180
ccgggttggt cggggcgggg cggggctcgg gggaccgggc tgacctcggg gtccgggcca 240
g                                     241
```

<210> 108
 <211> 241
 <212> DNA
 <213> homo sapiens

<400> 108

```
gtgagtgacc cgggcccggg gcgcaggta cgaacctca tccccacgg acgggccagg 60
tcgccacag tctccgggtc cgagatccgc cccgaagccg cgggaccccg agacccttgc 120
cccgggagag gccagggcgc cttaccggg ttcatcttc agtttaggcc aaaaatcccc 180
ccgggttggt cggggcgggg cggggctcgg gggaccgggc tgacctcggg gtccgggcca 240
g                                     241
```

<210> 109
 <211> 241
 <212> DNA
 <213> homo sapiens

<400> 109

```
gtgagtgacc cgggcccggg gcgcaggta cgaacctca tccccacgg acgggccagg 60
tcgccacag tctccgggtc cgagatccgc cccgaagccg cgggaccccg agacccttgc 120
cccgggagag gccagggcgc cttaccggg ttcatcttc agtttaggcc aaaaatcccc 180
ccgggttggt cggggcgggg cggggctcgg gggaccgggc tgacctcggg gtccgggcca 240
g                                     241
```

<210> 110
<211> 241
<212> DNA
<213> homo sapiens

<400> 110

```
gtgagtgacc ccggcccggg gcgcaggtea cgacccctca tccccacgg acgggccagg 60
tcgccacag tctccgggtc cgagatccgc cccgaagccg cgggaccccg agacccttgc 120
cccgggagag gccagggcgc cttaccggg ttccatttc agtttaggcc aaaaatcccc 180
ccgggttggt cggggcgggg cggggctcgg gggaccgggc tgacctcggg gtccgggcca 240
g                                     241
```

<210> 111
<211> 241
<212> DNA
<213> homo sapiens

<400> 111

```
gtgagtgacc ccggcccggg gcgcaggtea cgacctctca tccccacgg acgggccggg 60
tcgccacag tctccgggtc cgagatccac cccgaagccg cgggaccccg agacccttgc 120
cccgggagag gccagggcgc cttaccggg ttccatttc agtttaggcc aaaaatcccc 180
ccgggttggt cggggcgggg cggggctcgg gggaccgggc tgacctcggg gtccgggcca 240
g                                     241
```

<210> 112
<211> 241
<212> DNA
<213> homo sapiens

<400> 112

```
gtgagtgacc ccggcccggg gcgcaggtea cgacctctca tccccacgg acgggccggg 60
tcgccacag tctccgggtc cgagatccac cccgaagccg cgggaccccg agacccttgc 120
cccgggagag gccagggcgc cttaccggg ttccatttc agtttaggcc aaaaatcccc 180
ccgggttggt cggggccgga cggggctcgg gggactgggc tgacctggg gtcggggcca 240
g                                     241
```

<210> 113
<211> 241
<212> DNA
<213> homo sapiens

<400> 113

```
gtgagtgacc ccggcccggg gcgcaggtea cgacctctca tccccacgg acgggccggg 60
tcgccacag tctccgggtc cgagatccac cccgaagccg cgggaccccg agacccttgc 120
cccgggagag gccagggcgc cttaccggg ttccatttc agtttaggcc aaaaatcccc 180
ccgggttggt cggggccgga cggggctcgg gggactgggc tgacctggg gtcggggcca 240
```

g

241

<210> 114
<211> 241
<212> DNA
<213> homo sapiens

<400> 114

gtgagtgacc ccagccccggg gcgcaggtea cgacctctca tccccacgg acgggccagg 60
tcaccacag tctccgggtc cgagatccac cccgaagccg cgggacccc agacccttgc 120
cccgggagag gccagggcgc ctttaccggg ttctatttc agtttaggcc aaaaatcccc 180
ccgggttggt cggggccgga cggggctcgg gggactgggc tgaccgtggg gtcggggcca 240
g 241

<210> 115
<211> 241
<212> DNA
<213> homo sapiens

<400> 115

gtgagtgacc ccgccccggg gcgcaggtea cgacctctca tccccacgg acgggccagg 60
tcgcccacag tctccgggtc cgagatccac cccgaagccg cgggacccc agacccttgc 120
cccgggagag gccagggcgc ctttaccggg ttctatttc agtttaggcc aaaaatcccc 180
ccgggttggt cggggccgga cggggctcgg gggactgggc tgaccgtggg gtcggggcca 240
g 241

<210> 116
<211> 241
<212> DNA
<213> homo sapiens

<400> 116

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cccgggagag gccagggcgc ctttaccggg ttctatttc agtttaggcc aaaaatcccc 180
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<211> 241
<212> DNA
<213> homo sapiens

<400> 117

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cccgggagag gccagggcgc cttaccggg ttcatcttc agtttaggcc aaaaatcccc 180
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<212> DNA
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cccgggagag gccagggcgc cttaccggg ttcatcttc agtttaggcc aaaaatcccc 180
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<210> 119
<211> 241
<212> DNA
<213> homo sapiens

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cccgggagag gccagggcgc cttaccggg ttcatcttc agtttaggcc aaaaatcccc 180
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<210> 121
<211> 241
<212> DNA
<213> homo sapiens

<400> 121

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<210> 122
 <211> 241
 <212> DNA
 <213> homo sapiens

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<210> 123
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<210> 124
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 <211> 241
 <212> DNA
 <213> homo sapiens

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<210> 126

<211> 241

<212> DNA

<213> homo sapiens

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<211> 241

<212> DNA

<213> homo sapiens

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<211> 241

<212> DNA

<213> homo sapiens

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<212> DNA

<213> homo sapiens

<400> 129

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cccgggagag gccagggcgc cttaccggg ttcatcttc agtttaggcc aaaaatcccc   180
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<210> 130

<211> 241

<212> DNA

<213> homo sapiens

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cccgggagag gccagggcgc cttaccggg ttcatcttc agtttaggcc aaaaatcccc   180
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<210> 131

<211> 241

<212> DNA

<213> homo sapiens

<400> 131

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cccgggagag gccagggcgc cttaccggg ttcatcttc agtttaggcc aaaaatcccc   180
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<210> 132

<211> 241

<212> DNA

<213> homo sapiens

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cccgggagag gccagggcgc cttaccggg ttcatcttc agtttaggcc aaaaatcccc   180
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<210> 133

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cccgggagag gcccgaggcg ctttagccgg ttcatcttc agtttaggcc aaaaatcccc   180
ccgggtgggt cggggcgggg cggggctcgg gggaccgggc tgaccgctgg gtcggggcca   240
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<211> 600

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<213> homo sapiens

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<211> 600

<212> DNA

<213> homo sapiens

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<211> 600

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<210> 146

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<400> 146

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<211> 15

<212> DNA

<213> homo sapiens

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cccggttca ttttc

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<210> 245

<211> 20

<212> DNA

<213> homo sapiens

<400> 245

cttcacattc cgtgtctct

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<210> 246

<211> 129

<212> DNA

<213> homo sapiens

<400> 246

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cgccccag 129

<210> 247
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<212> DNA
<213> homo sapiens

<400> 247
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acccccag 128

<210> 248
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<212> DNA
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gccccag 128

<210> 249
<211> 129
<212> DNA
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ggccccag 129

<210> 250
<211> 128
<212> DNA
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gccccag 128

<210> 251
<211> 128
<212> DNA
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<400> 251

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gccccag 128

<210> 252

<211> 129

<212> DNA

<213> homo sapiens

<400> 252

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cgccccag 129

<210> 253

<211> 128

<212> DNA

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<400> 253

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gccccag 128

<210> 254

<211> 129

<212> DNA

<213> homo sapiens

<400> 254

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cgccccag 129

<210> 255

<211> 129

<212> DNA

<213> homo sapiens

<400> 255

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cgccccag 129

<210> 256

<211> 129

<212> DNA

<213> homo sapiens

<400> 256

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cgccccag 129
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<210> 257

<211> 129

<212> DNA

<213> homo sapiens

<400> 257

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cgccccag 129
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<210> 258

<211> 128

<212> DNA

<213> homo sapiens

<400> 258

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gccccag 128
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<210> 259

<211> 128

<212> DNA

<213> homo sapiens

<400> 259

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gccccag 128
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<210> 260

<211> 128

<212> DNA

<213> homo sapiens

<400> 260

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gccccccag

128

<210> 261

<211> 129

<212> DNA

<213> homo sapiens

<400> 261

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gccccccag 129

<210> 262

<211> 129

<212> DNA

<213> homo sapiens

<400> 262

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tgccccccag 129

<210> 263

<211> 129

<212> DNA

<213> homo sapiens

<400> 263

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tgccccccag 129

<210> 264

<211> 129

<212> DNA

<213> homo sapiens

<400> 264

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cgccccccag 129

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<211> 129

<212> DNA

<213> homo sapiens

<400> 265

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cgccccag 129

<210> 266

<211> 129

<212> DNA

<213> homo sapiens

<400> 266

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cgccccag 129

<210> 267

<211> 129

<212> DNA

<213> homo sapiens

<400> 267

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cgccccag 129

<210> 268

<211> 129

<212> DNA

<213> homo sapiens

<400> 268

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cgccccag 129

<210> 269

<211> 129

<212> DNA

<213> homo sapiens

<400> 269

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cgccccag 129

<210> 270

<211> 129
<212> DNA
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<400> 270

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cgccccag 129

<210> 271
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<212> DNA
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<400> 271

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gccccag 128

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<400> 272

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ggggcgcagga cctgaggagc cgcgccggga ggagggtcgg gcggtctca gccctcctc 120
gccccag 128

<210> 273
<211> 128
<212> DNA
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<400> 273

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ggggcgcagga cctgaggagc cgcgccggga ggagggtcgg gcggtctca gccctcctc 120
gccccag 128

<210> 274
<211> 128
<212> DNA
<213> homo sapiens

<400> 274

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gggcgagga cctgaggagc cgcgccggga ggagggtcgg gcgggtctca gcccctctc 120
gccccag 128

<210> 275

<211> 128

<212> DNA

<213> homo sapiens

<400> 275

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gggcgagga cccggggagc cgcgccggga ggagggtcgg gcgggtctca gctcctctc 120
gccccag 128

<210> 276

<211> 129

<212> DNA

<213> homo sapiens

<400> 276

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gggcgagga accggggag cgcgccggga aggagggtcg gcgggtctc agcccctct 120
cgccccag 129

<210> 277

<211> 128

<212> DNA

<213> homo sapiens

<400> 277

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gggcgagga cccggggagc cgcgccggga ggagggtcgg gcgggtctca gctcctctc 120
gccccag 128

<210> 278

<211> 128

<212> DNA

<213> homo sapiens

<400> 278

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gggcgagga cccggggagc cgcgccggga ggagggtcgg gcgggtctca gctcctctc 120
gccccag 128

<210> 279

<211> 128

<212> DNA

<213> homo sapiens

<400> 279

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ggggcgagga cctgaggagc cgcgccggga ggagggtcgg gcgggtctca gcccctctc 120
acccccag 128

<210> 280

<211> 129

<212> DNA

<213> homo sapiens

<400> 280

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ggggcgagga accgggggag ccgcgccggg aggagggtcg ggcgggtctc agcccctct 120
cgccccag 129

<210> 281

<211> 129

<212> DNA

<213> homo sapiens

<400> 281

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ggggcgagga accgggggag ccgcgccggg aggagggtcg ggcgggtctc agcccctct 120
cgccccag 129

<210> 282

<211> 128

<212> DNA

<213> homo sapiens

<400> 282

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ggggcgagga cccggggagc cgcgccggga ggagggtcgg gcgggtctca gcccctctc 120
gccccag 128

<210> 283

<211> 129

<212> DNA

<213> homo sapiens

<400> 283

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ggggcgagga accgggggag ccgcgccggg aggagggtcg ggcgggtctc agcccctct 120
cgccccag 129

<210> 284
<211> 129
<212> DNA
<213> homo sapiens

<400> 284

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ggggctcagg acccggggag ccgcgccggg aggagggtcg ggcggtctc agccctcct 120
cgccccag 129

<210> 285
<211> 128
<212> DNA
<213> homo sapiens

<400> 285

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ggggcgagga cctgaggagc cgcccgaggg ggagggtcgg gcgggtctca gcccctcctc 120
acccccag 128

<210> 286
<211> 128
<212> DNA
<213> homo sapiens

<400> 286

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ggggcgagga cccggggagc cgcccgaggg ggagggtcgg gcgggtctca gcccctcctc 120
gccccag 128

<210> 287
<211> 128
<212> DNA
<213> homo sapiens

<400> 287

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ggggcgagga cccggggagc cgcccgaggg ggagggtcgg gcgggtctca gcccctcctc 120
gccccag 128

<210> 288
<211> 129
<212> DNA
<213> homo sapiens

<400> 288

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ggggcgagg acctgaggag ccgcgccggg aggagggtcg ggcgggtctc agccctctct 120
cgccccag 129

<210> 289
<211> 129
<212> DNA
<213> homo sapiens

<400> 289
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cgccccag 129

<210> 290
<211> 129
<212> DNA
<213> homo sapiens

<400> 290
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ggggcgagg acctgaggag ccgcgccggg aggagggtcg ggcgggtctc agccctctct 120
cgccccag 129

<210> 291
<211> 129
<212> DNA
<213> homo sapiens

<400> 291
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cgccccag 129

<210> 292
<211> 128
<212> DNA
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<400> 292
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gccccag 128

<210> 293
<211> 128

<212> DNA

<213> homo sapiens

<400> 293

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gggcgcagga cccggggagc cgcgccggga ggagggtctg gcgggtctca gccctctc 120
gccccag                                     128

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<210> 294

<211> 128

<212> DNA

<213> homo sapiens

<400> 294

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gccccag                                     128

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<210> 295

<211> 128

<212> DNA

<213> homo sapiens

<400> 295

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gggcgcagga cccggggagc cgcgccggga ggagggtctg gcgggtctca gccctctc 120
gccccag                                     128

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<210> 296

<211> 129

<212> DNA

<213> homo sapiens

<400> 296

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ggggcgcagg acctgaggag ccgcgccggg aggagggtcg ggccgggtctc agcccctct 120
cgccccag                                     129

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<210> 297

<211> 128

<212> DNA

<213> homo sapiens

<400> 297

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gggcgcagga cccggggagc cgcgccggga ggagggtctg gcgggtctca gccctctc 120

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gccccag

128

<210> 298

<211> 128

<212> DNA

<213> homo sapiens

<400> 298

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 gccccag 128

<210> 299

<211> 128

<212> DNA

<213> homo sapiens

<400> 299

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 gccccag 128

<210> 300

<211> 245

<212> DNA

<213> homo sapiens

<400> 300

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 ctgaccggc gagagccca ggcgcgttta cccggttca tttcagttg aggccaaaat 180
 cccgcgggt tggtcggggc ggggcggggc tcgggggacg gggctgaccg cggggtcggg 240
 gccag 245

<210> 301

<211> 245

<212> DNA

<213> homo sapiens

<400> 301

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 ctgaccggc gagagccca ggcgcgttta cccggttca tttcagttg aggccaaaat 180
 cccgcgggt tggtcggggc ggggcggggc tcgggggact gggctgaccg cggggccggg 240
 gccag 245

<210> 302
 <211> 246
 <212> DNA
 <213> homo sapiens

<400> 302

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ctcgaccggc gagagcccca ggcgcgttta cccggtttca tttcagttg aggccaaaat 180
ccccgcgggt tggtcggggc ggggcggggc tcggggggac ggggctgacc gcggggccgg 240
ggccag 246
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<210> 303
 <211> 245
 <212> DNA
 <213> homo sapiens

<400> 303

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tcgccccgag tctccgggtc cgagatccac ctccctgagg ccgcgggacc cgcccagacc 120
ctcgaccggc gagagcccca ggcgcgttta cccggtttca tttcagttg aggccaaaat 180
ccccgcgggt tggtcggggc ggggcggggc tcggggggac ggggctgacc cgggggccgg 240
gccag 245
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<210> 304
 <211> 246
 <212> DNA
 <213> homo sapiens

<400> 304

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ctcgaccggc gagagcccca ggcgcgttta cccggtttca tttcagttg aggccaaaat 180
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ggccag 246
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<210> 305
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<400> 305

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ctcgaccggc gagagcccca ggcgcgttta cccggtttca tttcagttg aggccaaaat 180
ccccgcgggt tgggcggggc ggggcggggc tcggggggac tgggctgacc gcggggccgg 240
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ggccag

246

<210> 306
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<400> 306

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 ctgaccggc gagagccca ggcggttta cccggttca tttcagttg aggccaaaat 180
 cccgcgggt tggcggggc gggcggggc tcgggggac ggggctgacc gggggcctg 240
 ggccag 246

<210> 307
 <211> 245
 <212> DNA
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<400> 307

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 ctgaccggc gagagccca ggcggttta cccggttca tttcagttg aggccaaaat 180
 cccgcgggt tggcggggc gggcggggc tcgggggac ggggctgacc gggggcctg 240
 gccag 245

<210> 308
 <211> 245
 <212> DNA
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<400> 308

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 ctgaccggc gagagccca ggcggttta cccggttca tttcagttg aggccaaaat 180
 cccgcgggt tggcggggc gggcggggc tcgggggac ggggctgacc gggggcctg 240
 gccag 245

<210> 309
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ctcgaccggc gagagcccca ggcgcgttta cccggtttca tttcagttg aggccaaaat 180
 ccccgcggtt tggtcggggc ggggcggggc tcgggggacg gggctgaccg cggggcctgg 240
 gccag 245

<210> 310
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 ctcgaccggc gagagcccca ggcgcgttta cccggtttca tttcagttg aggccaaaat 180
 ccccgcggtt tggtcggggc ggggcggggc tcgggggacg gggctgaccg cggggcctgg 240
 gccag 245

<210> 311
 <211> 245
 <212> DNA
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<400> 311
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 gccag 245

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 ctcgaccggc gagagcccca ggcgcgttta cccggtttca tttcagttg aggccaaaat 180
 ccccgcggtt tggtcggggc ggggcggggc tcgggggacg gggctgaccg cggggcctg 240
 gccag 246

<210> 313
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<400> 313

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ctcgaccggc gagagccca ggcgcgttta ccggtttca tttcagttg aggccaaaat 180
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ggccag 246

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<210> 314
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 <212> DNA
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ctcgaccggc gagagccca ggcgcgttta ccggtttca tttcagttg aggccaaaat 180
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ggccag 246

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<210> 315
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 <212> DNA
 <213> homo sapiens

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cgaccggcga gagcccaggc gcgtttacc ggtttcatt tcagttgagg ccaaaatccc 180
cgcgggttgg tcggggcggg gcggggctcg gggggacggg gctgaccgc ggggcggggc 240
cag 243

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<210> 316
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 <212> DNA
 <213> homo sapiens

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cgaccggcga gagcccaggc gcgtttacc ggtttcatt ttcagttgag gccaaaatcc 180
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ccag 244

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<210> 317
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 <212> DNA
 <213> homo sapiens

<400> 317

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cgaccggcga gagccccagg cgcgtttacc cggtttcatt ttcagttgag gccaaaatcc    180
ccgcgggttg gtcggggcgg ggccggggctc ggggggacgg ggctgaccgc gggggcgggg    240
ccag                                     244

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<210> 318

<211> 244

<212> DNA

<213> homo sapiens

<400> 318

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cgaccggcga gagccccagg cgcgtttacc cggtttcatt ttcagttgag gccaaaatcc    180
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<210> 319

<211> 244

<212> DNA

<213> homo sapiens

<400> 319

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cgaccggcga gagccccagg cgcgtttacc cggtttcatt ttcagttgag gccaaaatcc    180
ccgcgggttg gtcggggcgg ggccggggctc ggggggacgg ggctgaccgc gggggcgggg    240
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<210> 320

<211> 245

<212> DNA

<213> homo sapiens

<400> 320

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ctcgaccggc gagagcccca ggccggttta ccggtttca tttcagttg agggccaaat    180
ccccgcgggt tggtcggggc ggggcggggc tcgggggacg gggctgaccg cggggcgggg    240
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<210> 321

<211> 245

<212> DNA

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<400> 321

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ctcgaccggc gagagcccca ggcgcgttta cccggtttca tttcagttg aggccaaaat  180
ccccgcgggt tggtcggggc ggggcggggc tcgggggacg gggctgaccg cggggccggg  240
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<210> 322

<211> 245

<212> DNA

<213> homo sapiens

<400> 322

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ctcgaccggc gagagcccca ggcgcgttta cccggtttca tttcagttg aggccaaaat  180
ccccgcgggt tggtcggggc ggggcggggc tcgggggacg gggctgaccg cggggccggg  240
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<210> 323

<211> 245

<212> DNA

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<400> 323

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ctcgaccggc gagagcccca ggcgcgttta cccggtttca tttcagttg aggccaaaat  180
ccccgcgggt tggtcggggc ggggcggggc tcgggggacg gggctgaccg cggggccggg  240
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gccag                                     245

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ctcgaccggc gagagcccca ggcgcgttta cccggtttca tttcagttg aggccaaaat  180
ccccgcgggt tggtcggggc ggggcggggc tcggggggac ggggctgacc gcggggccgg  240
ggccag                                     246
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<210> 326
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ctcgaccggc gagagcccca ggcgcgttta cccggtttca tttcagttg aggccaaaat  180
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ggccag                                     246
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ctcgaccggc gagagcccca ggcgcgttta cccggtttca tttcagttg aggccaaaat  180
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ggccag                                     246
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ctcgaccggc gagagcccca ggcgcgttta cccggtttca tttcagttg aggccaaaat  180
ccccgcgggt tggtcggggc ggggcggggc tcggggggac ggggctgacc gcggggccgg  240
ggccag                                     246
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 ccag 244

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ggccag

246

<210> 333

<211> 246

<212> DNA

<213> homo sapiens

<400> 333

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<211> 246

<212> DNA

<213> homo sapiens

<400> 334

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<211> 243

<212> DNA

<213> homo sapiens

<400> 335

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<212> DNA

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<400> 336

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21

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243

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ccccgcgggt tggtcggggc ggggcggggc tcgggggact gggctgaccg cggggccggg  240
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<400> 342

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ccccgcgggt tggtcggggc ggggcggggc cgggggacgg gggctgaccg ggggcctggg  240
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ctcgaccggc gagagcccca ggcgcgttta cccggtttca tttcagttg aggccaaaat  180
ccccgcgggt tggtcggggc ggggcggggc tcgggggacg gggctgaccg cggggcctgg  240
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<210> 344

<211> 246

<212> DNA

<213> homo sapiens

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<400> 348

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 ggccag 246

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gcatgagttt tctgagttt cctctgaggg cccctcttc tctctagaca attaaggaat   180
gacgtctctg aggaatgga gggaagaca gtccttagaa tactgatcag ggggtccct   240
tgaccctgc agcagccttg ggaacctga ctttctct caggccttgt tcttgcctc   300
acactcagtg tgttggggc tctgattcca gcacttctg gtcactttac ctcactcag  360
atcaggagca gaagtccctg ttccccgct agagactcga actttccaa gaataggaga  420
ttatcccagg tgcctgcgtc caggctgggt tctgggttct gtccccctc cccacccag  480
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<210> 380

<211> 575

<212> DNA

<213> homo sapiens

<400> 380

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gcatgagttt tctgagttt cctctgaggg cccctcttc tctctaggac aattaaggga   180
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attatcccag gtgcctgcgt ccagctgggt gtctgggttc tgtccccct cccacccca  480
ggtgtcctgt ccattctcag gctgtcaca tgggtgttcc tagggtgtcc catgagagat  540
gcaaagccc tgaatttct gacttctcc atcag                               575

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<210> 381
 <211> 572
 <212> DNA
 <213> homo sapiens

<400> 381

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gcatgagttt tcttgagttt cctctgaggg cccctcttc tctctagaca attaagggat 180
gacgtctctg aggaaatgga ggggaagaca gtcctagaa tac gatcag ggggtccctt 240
tgaccctgc agcagccttg ggaaccgtga ctttctct caggccttgt tctctgcctc 300
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atcaggagca gaagtcctg tccccgctc agagactcga acttccaat gaataggaga 420
ttatcccagg tgcctgcac cgtgtgtgtc tgggttctgt gcccctccc caccacaggt 480
gtctgtcca ttctcaggct ggtcacatgg gtgttcctag ggtgtgccat gagagatgca 540
aagcgctga attttctgac tcttccatc ag 572
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<210> 382
 <211> 572
 <212> DNA
 <213> homo sapiens

<400> 382

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gcatgagttt tcttgagttt cctctgaggg cccctcttc tctctagaca attaaggaat 180
gacgtctctg aggaaatgga ggggaagaca gtcctagaa tactgatcag ggggtccctt 240
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atcaggagca gaagtcctg tccccgctc agagactcga acttccaat gaataggaga 420
ttatcccagg tgcctgcac cgtgtgtgtc tgggttctgt gcccctccc caccacaggt 480
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<210> 383
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 <212> DNA
 <213> homo sapiens

<400> 383

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gcatgagttt tcttgagttt cctctgaggg cccctcttc tctctagaca attaagggat 180
gacgtctctg aggaaatgga ggggaagaca gtcctagaa tactgatcag ggggtccctt 240
tgaccctgc agcagccttg ggaaccgtga ctttctct caggccttgt tctctgcctc 300
acactcagtg tgtttggggc tctgattcca gcacttctga gtcactttac ctccactcag 360
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atcaggagca gaagtcctg tccccgctc agagactcga actttccaat gaataggaga 420
ttatcccagg tgectgcac cgctgggtgc tgggttctgt gcccctccc caccacaggt 480
gtcctgtcca ttctcaggct ggtcacatgg gtggtcctag ggtgtgccat gagagatgca 540
aagcgctga attttctgac tcttcccatc ag 572

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<210> 384
 <211> 575
 <212> DNA
 <213> homo sapiens

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<400> 384
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gcatgagttt tcttgagttt cctctgaggg cccccttc tctctaggac aattaaggga 180
tgacgtctct gaggaatgg aggggaagac agtcctaga atactgatca ggggtcccct 240
ttgacccctg cagcagcctt gggaaccgtg acttttctc tcaggccttg ttctctgect 300
cacactcagt gtgttgggg ccttgattcc agcacttctg agtcacttta cctccactca 360
gatcaggagc agaagtcctt gtccccgct cagagactcg aactttccaa tgaataggag 420
attatcccag gtgcctgcgt ccaggctggt gtctgggttc tgtccccctt cccacccca 480
ggtgtcctgt ccattctcag gctggtcaca tgggtggtcc tagggtgtcc catgagagat 540
gcaaagcgcc tgaattttct gactcttccc atcag 575

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<210> 385
 <211> 575
 <212> DNA
 <213> homo sapiens

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<400> 385
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gcatgagttt tcttgagttt cctctgaggg cccccttc tctctaggac aattaaggga 180
tgacgtctct gaggaatgg aggggaagac agtcctaga atactgatca ggggtcccct 240
ttgacccctg cagcagcctt gggaaccgtg acttttctc tcaggccttg ttctctgect 300
cacactcagt gtgttgggg ccttgattcc agcacttctg agtcacttta cctccactca 360
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attatcccag gtgcctgcgt ccaggctggt gtctgggttc tgtccccctt cccacccca 480
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gcaaagcgcc tgaattttct gactcttccc atcag 575

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<210> 386
 <211> 573
 <212> DNA
 <213> homo sapiens

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<400> 386
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gcatgagttt tctgagttt cctctgagg cccctcttc tcttagaca attaaggga 180
gacgtctctg aggaaatgga ggggaagaca gtcctagaa tactgatcag ggggccctt 240
tgaccctgc agcagcctg ggaaccgtga cttctctc aggccttgt ctctgcctca 300
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tatcccaggt gcctgcgtcc aggtctgtgt ctgggttctg tgcccttcc ccaccacagg 480
tgtctgtcc attctcaggc tggtcacatg ggtgtccta ggtgtccca tgagagatgc 540
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<210> 387

<211> 575

<212> DNA

<213> homo sapiens

<400> 387

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gcatgagttt tctgagttt cctctgagg cccctcttc tcttaggac aattaaggga 180
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attatcccag gtgcctgcgt ccaggctggt gtctgggttc tgtgccctt cccaccacca 480
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<210> 388

<211> 575

<212> DNA

<213> homo sapiens

<400> 388

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gcatgagttt tctgagttt cctctgagg cccctcttc tcttaggac aattaaggga 180
tgacgtctct gaggaatgg aggggaagac agtcctaga atactgatca ggggtccct 240
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ggtgtctgt ccattctcag gctggtcaca tgggtggtcc tagggtgtcc catgagagat 540
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<210> 389

<211> 575

<212> DNA

<213> homo sapiens

<400> 389

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attatcccag gtgcctgcgt ccaggctggg gtctgggttc tgtgccctt cccacacca 480
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<211> 576

<212> DNA

<213> homo sapiens

<400> 390

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gcatgagttt tctgagttt cctctgagg cccctcttc tctctaggac aattaaggga 180
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gattatccca ggtgcctgcg tccaggctgg gtctgggttc ctgtgccct tccccacccc 480
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<400> 391

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gcatgagttt tctgagttt cctctgagg cccctcttc tctctaggac aattaaggga 180
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gcaaagcgcc tgaattttct gactcttccc atcag 575

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gcatgagttt tctgagttt cctctgaggg cccctcttc tctctaggac aattaaggga 180

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<213> homo sapiens

<400> 396

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<211> 575

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<400> 397

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<400> 398

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<400> 401

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<400> 412
ggtctcagcc cctctca 18

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<210> 419
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<213> homo sapiens

<400> 419
ggccctcaga ggaaactcg 19

<210> 420
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<400> 420
aggcctgaga ggaaaagtca t 21

<210> 421
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gatcagtatt ctagggactg a 21

<210> 423
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<400> 442

WO 99/07883

PCT/CA98/00768

tcccactcca tgaggtattt c

21

WHAT IS CLAIMED IS:

1. A method of determining the HLA-B Class I group type of a subject comprising the following steps:

(i) combining a group-specific untranslated region primer pair with a target DNA sample from the subject under conditions such that primer-based amplification of the target DNA may occur; and

(ii) determining whether a nucleic acid product is produced by the amplification;

wherein the ability of the primer pair to produce a nucleic acid product is associated with a particular HLA group type.

2. The method of claim 1, further comprising the step of (iii) determining the nucleic acid sequence of the nucleic acid product of step (ii).

3. The method of claim 1, wherein the primer pair comprises one or more oligonucleotide primers selected from the group consisting of E1-B121m17, E1-B129, E1-B130, E1-B136, E1-B182, I1-B145, I1-B154m, I1-B167, I1-B168, I1-B169, I1-B170, I1-B171, I1-B172, I1-B173, I1-B174, I1-B175, I1-B326, I1-B331, I1-B346, I3-B126, I3-B147, I3-B164, I3-B165, I3-B166, I3-B187, I3-B212, I3-B305, I3-B319, I3-B320, I3-B321, I3-B323, I3-B332, I3-B335, I3-B337, I3-B342, I3-B347, I3-B348, and I3-B349.

4. The method of claim 1, wherein the primer pair is selected from the group of pairs consisting of I1-B174 and I3-B305; I1-B167 and I3-B323; I1-B175 and I3-B319; I1-B145 and I3-B321; E1-B121m17 and I3-B147; I1-B154m and I3-B164; E1-B182 and I3-B348; I1-B168 and I3-B212; I1-B326 and I3-B165; I1-B167 and I3-B320, I1-B172 and I3-B342; I1-B172 and I3-B323; I1-B174 and I3-B323; I1-B170 and I3-B126; I1-B326 and I3-B348; I1-B331 and I3-B332; I1-B326 and I3-B337; I1-B326 and I3-B187; I1-B169 and I3-B166; I1-B171 and I3-B347; I1-B173 and I3-B335; I1-B168 and I3-B212; I1-B346 and I3-B126; I3-B326 and I3-B126; I1-B167 and I3-B126; I1-B168 and I3-B126; E1-B129 and I3-B126; E1-B130 and I3-B126; E1-B182 and I3-B126; and E1-B136 and I3-B126.

5. A method of determining the HLA-B Class I allele type of a subject, wherein the group type of the subject is known, comprising the following steps:

(i) combining a group-specific untranslated region primer pair corresponding to the group type of the subject with a target DNA sample from the subject under conditions such that primer-based amplification of the target DNA may occur and a second nucleic acid product is produced; and

(ii) determining the nucleic acid sequence of the second nucleic acid product collected in step (i).

6. The method of claim 5, wherein the group-specific untranslated region primer pair used in step (i) comprises one or more oligonucleotide primers selected from the group consisting of E1-B121m17, E1-B129, E1-B130, E1-B136, E1-B182, I1-B145, I1-B154m, I1-B167, I1-B168, I1-B169, I1-B170, I1-B171, I1-B172, I1-B173, I1-B174, I1-B175, I1-B326, I1-B331, I1-B346, I3-B126, I3-B147, I3-B164, I3-B165, I3-B166, I3-B187, I3-B212, I3-B305, I3-B319, I3-B320, I3-B321, I3-B323, I3-B332, I3-B335, I3-B337, I3-B342, I3-B347, I3-B348, and I3-B349.

7. The method of claim 5, wherein the group-specific untranslated region primer pair used in step (i) is selected from the group of oligonucleotide primer pairs consisting of I1-B174 and I3-B305; I1-B167 and I3-B323; I1-B175 and I3-B319; I1-B145 and I3-B321; E1-B121m17 and I3-B147; I1-B154m and I3-B164; E1-B182 and I3-B349; I1-B168 and I3-B212; I1-B326 and I3-B165; I1-B167 and I3-B320; I1-B172 and I3-B342; I1-B172 and I3-B323; I1-B174 and I3-B323; I1-B170 and I3-B126; I1-B326 and I3-B348; I1-B331 and I3-B332; I1-B326 and I3-B337; I1-B326 and I3-B187; I1-B169 and I3-B166; I1-B171 and I3-B347; I1-B173 and I3-B335; I1-B168 and I3-B212; I1-B346 and I3-B126; I3-B326 and I3-B126; I1-B167 and I3-B126; I1-B168 and I3-B126; E1-B129 and I3-B126; E1-B130 and I3-B126; E1-B182 and I3-B126; and E1-B136 and I3-B126.

8. A composition comprising a plurality of oligonucleotide primer pairs comprising one or more primers selected from the group consisting of E1-B121m17, E1-B129, E1-B130, E1-B136, E1-B182, I1-B145, I1-B154m, I1-B167, I1-B168, I1-B169, I1-B170, I1-B171, I1-B172, I1-B173, I1-B174, I1-B175, I1-B326, I1-B331, I1-B346, I3-B126, I3-B147, I3-B164, I3-B165, I3-B166, I3-B187, I3-B212, I3-B305, I3-B319, I3-B320, I3-B321, I3-B323, I3-B332, I3-B335, I3-B337, I3-B342, I3-

B347, I3-B348, and I3-B349.

9. A composition comprising an oligonucleotide primer selected from the group consisting of E1-B121m17, E1-B129, E1-B130, E1-B136, E1-B182, I1-B145, I1-B154m, I1-B167, I1-B168, I1-B169, I1-B170, I1-B171, I1-B172, I1-B173, I1-B174, I1-B175, I1-B326, I1-B331, I1-B346, I3-B126, I3-B147, I3-B164, I3-B165, I3-B166, I3-B187, I3-B212, I3-B305, I3-B319, I3-B320, I3-B321, I3-B323, I3-B332, I3-B335, I3-B337, I3-B342, I3-B347, I3-B348, and I3-B349.

10. A composition comprising an oligonucleotide primer pair selected from the group consisting of I1-B174 and I3-B305; I1-B167 and I3-B323; I1-B175 and I3-B319; I1-B145 and I3-B321; E1-B121m17 and I3-B147; I1-B154m and I3-B164; E1-B182 and I3-B349; I1-B168 and I3-B212; I1-B326 and I3-B165; I1-B167 and I3-B320; I1-B172 and I3-B342; I1-B172 and I3-B323; I1-B174 and I3-B323; I1-B170 and I3-B126; I1-B326 and I3-B348; I1-B331 and I3-B332; I1-B326 and I3-B337; I1-B326 and I3-B187; I1-B169 and I3-B166; I1-B171 and I3-B347; I1-B173 and I3-B335; I1-B168 and I3-B212; I1-B346 and I3-B126; I3-B326 and I3-B126; I1-B167 and I3-B126; I1-B168 and I3-B126; E1-B129 and I3-B126; E1-B130 and I3-B126; E1-B182 and I3-B126; and E1-B136 and I3-B126.

11. A kit comprising:

(a) a plurality of oligonucleotide group-specific untranslated region primer pairs comprising one or more primers selected from the group consisting of E1-B121m17, E1-B129, E1-B130, E1-B136, E1-B182, I1-B145, I1-B154m, I1-B167, I1-B168, I1-B169, I1-B170, I1-B171, I1-B172, I1-B173, I1-B174, I1-B175, I1-B326, I1-B331, I1-B346, I3-B126, I3-B147, I3-B164, I3-B165, I3-B166, I3-B187, I3-B212, I3-B305, I3-B319, I3-B320, I3-B321, I3-B323, I3-B332, I3-B335, I3-B337, I3-B342, I3-B347, I3-B348, and I3-B349;

and

(b) an enzyme for nucleotide chain extension.

12. A kit comprising:

(a) an oligonucleotide group-specific untranslated region primer selected from the group consisting of E1-B121m17, E1-B129, E1-B130, E1-B136, E1-B182, I1-B145, I1-B154m, I1-B167, I1-B168, I1-B169, I1-B170, I1-B171, I1-

B172, I1-B173, I1-B174, I1-B175, I1-B326, I1-B331, I1-B346, I3-B126, I3-B147, I3-B164, I3-B165, I3-B166, I3-B187, I3-B212, I3-B305, I3-B319, I3-B320, I3-B321, I3-B323, I3-B332, I3-B335, I3-B337, I3-B342, I3-B347, I3-B348, and I3-B349 ; and

(b) an enzyme for nucleotide chain extension.

13. A kit comprising:

(a) an oligonucleotide primer pair selected from the group consisting of I1-B174 and I3-B305; I1-B167 and I3-B323; I1-B175 and I3-B319; I1-B145 and I3-B321; E1-B121m17 and I3-B147; I1-B154m and I3-B164; E1-B182 and I3-B349; I1-B168 and I3-B212; I1-B326 and I3-B165; I1-B167 and I3-B320; I1-B172 and I3-B342; I1-B172 and I3-B323; I1-B174 and I3-B323; I1-B170 and I3-B126; I1-B326 and I3-B348; I1-B331 and I3-B332; I1-B326 and I3-B337; I1-B326 and I3-B187; I1-B169 and I3-B166; I1-B171 and I3-B347; I1-B173 and I3-B335; I1-B168 and I3-B212; I1-B346 and I3-B126; I3-B326 and I3-B126; I1-B167 and I3-B126; I1-B168 and I3-B126; E1-B129 and I3-B126; E1-B130 and I3-B126; E1B-182 and I3-B126; and E1B-136 and I3-B126; and

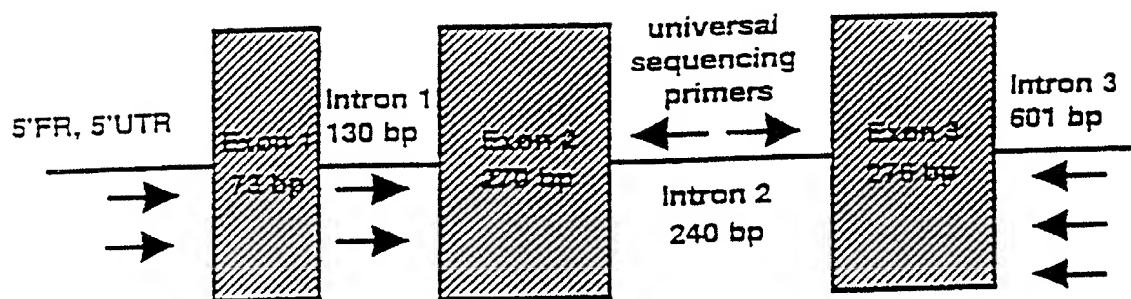
(b) an enzyme for nucleotide chain extension.

14. The kit of claim 13, further comprising:

(d) a sequencing primer selected from the group consisting of GGA TCT CGG ACC CGG AGA CTC G (SEQ ID NO:436); ACC CGG TTT CAT TTT CAG TTG (SEQ ID NO:437); TTT ACC CGG TTT CAT TTT CAG TT (SEQ ID NO:438); TCC CCA CTG CCC CTG GTA (SEQ ID NO:439); GGK CCA GGG TCT CAC A (SEQ ID NO:440); ATC TCG GAC CCG GAG ACT (SEQ ID NO:441); and TCC CAC TCC ATG AGG TAT TTC (SEQ ID NO:442).

Figure 1.

HLA class I Sequencing



group-specific non-coding region primers

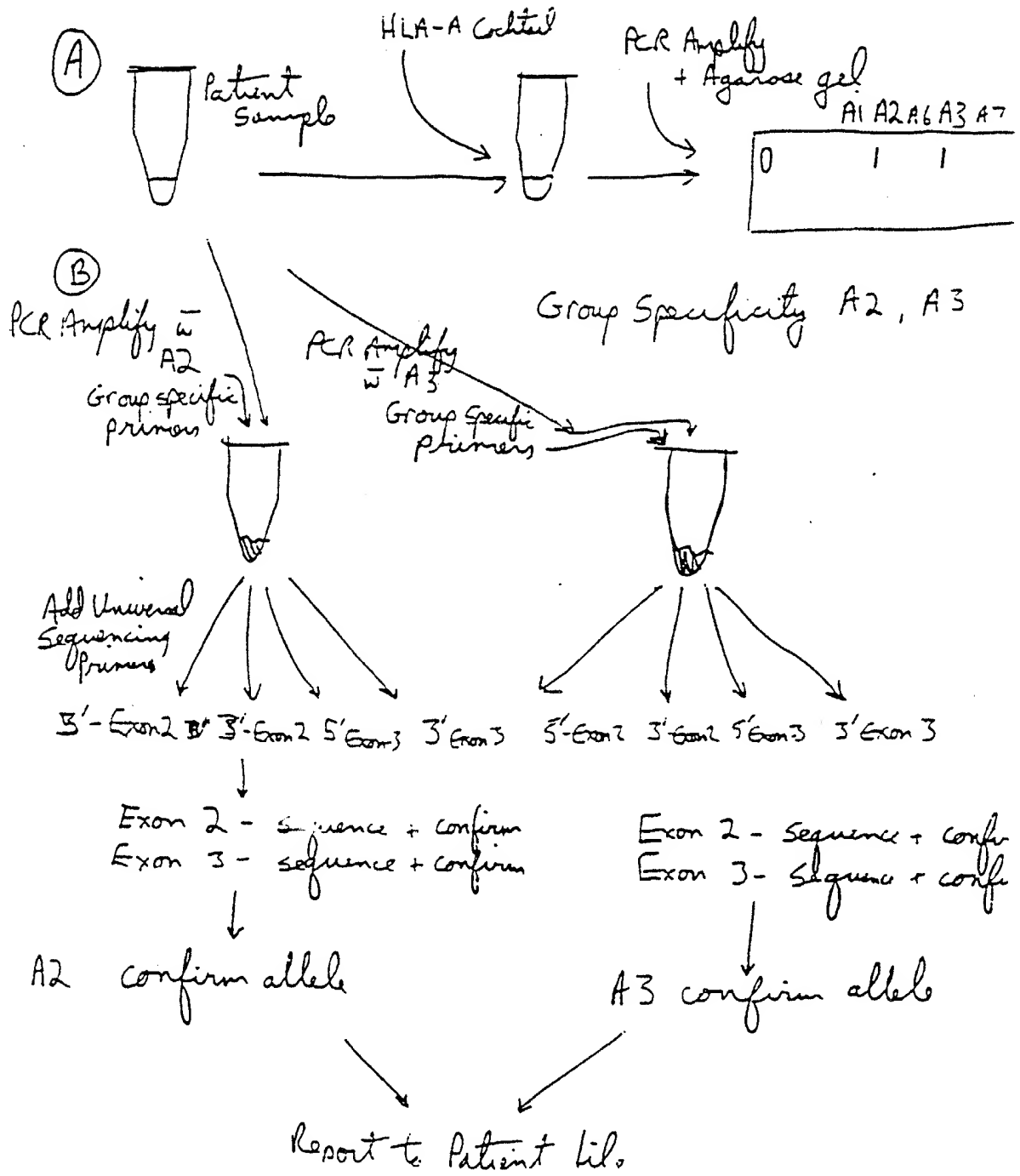


Figure 2(i)

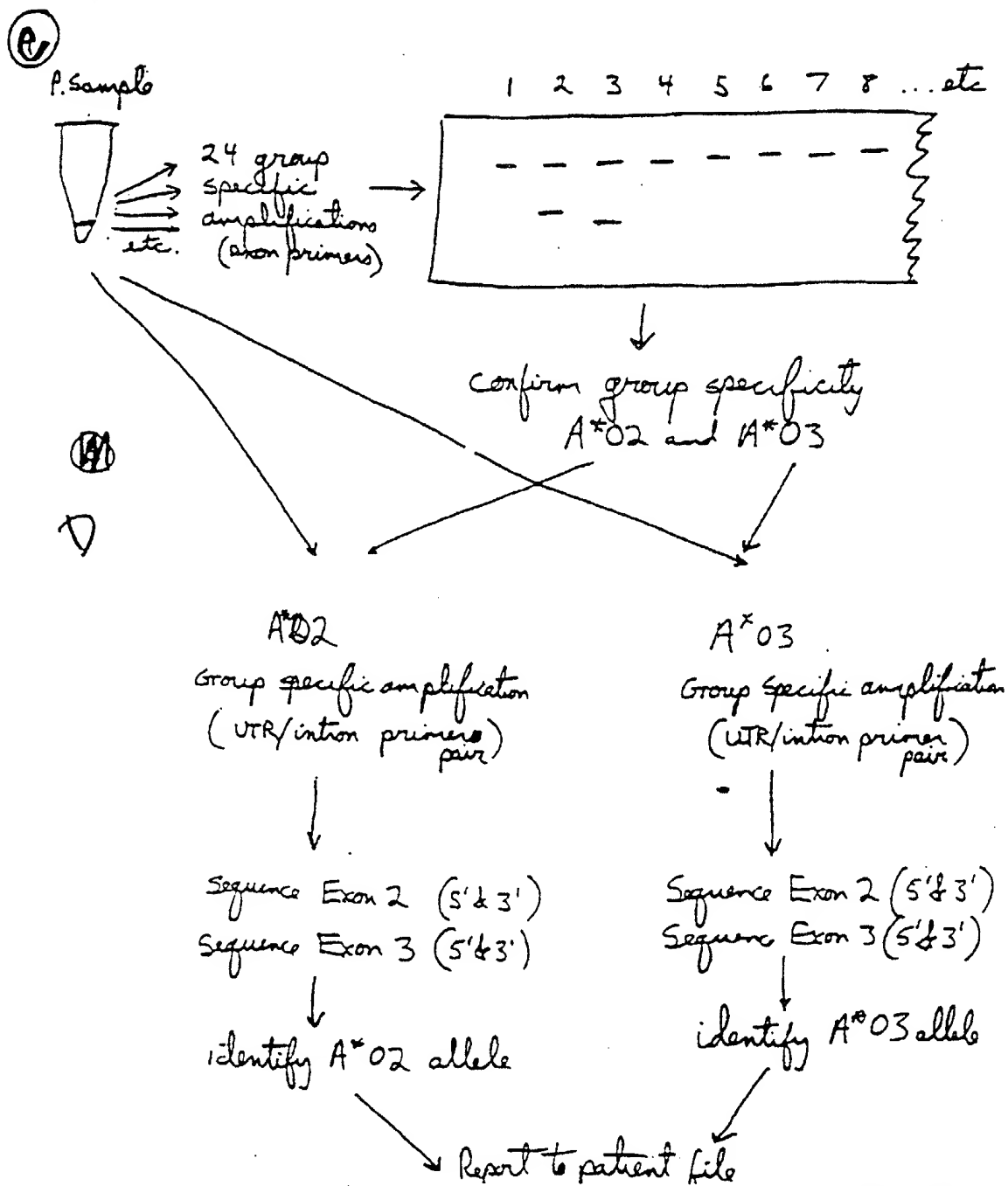


Figure 2(2)

Figure 3(1)

Abbildung 20

HLA-A 5' flanking region, Teil II

	110	120	130	140	150	160	170	180	190	200
Consensus	CTCTTACC	CAAGCC	AGACAC	CTCATAG	CTCTTCTG	CTGAGATG	ATATCC	CAACCT	CTCTCTTCTT	GTACGCGCTCA
A*0101
A*0301
A*1101
A*1102
A*1001
A*1002
A*1004
A*0201-11
A*0215
A*0217
A*6801
A*6802
A*6901
A*2101
A*2102
A*2103
A*2104
A*2105
A*2107
A*2501
A*2601
A*3102
A*4101
A*6601
A*6602
A*6603
A*2901
A*2902
A*31012
A*3101
A*3103
A*7101
A*7102
A*7403
A*8001

Abbildung 7a (c)

Figure 3(2)

Figure 3 (3)

BNSDOCID: <WO_9907883A1_|_>

Figure 3(4)

[illegible]

Abbildung 20 (Fortsetzung)

Figure 3(5)

8 / 29

III A-A Intron 2, Teil I

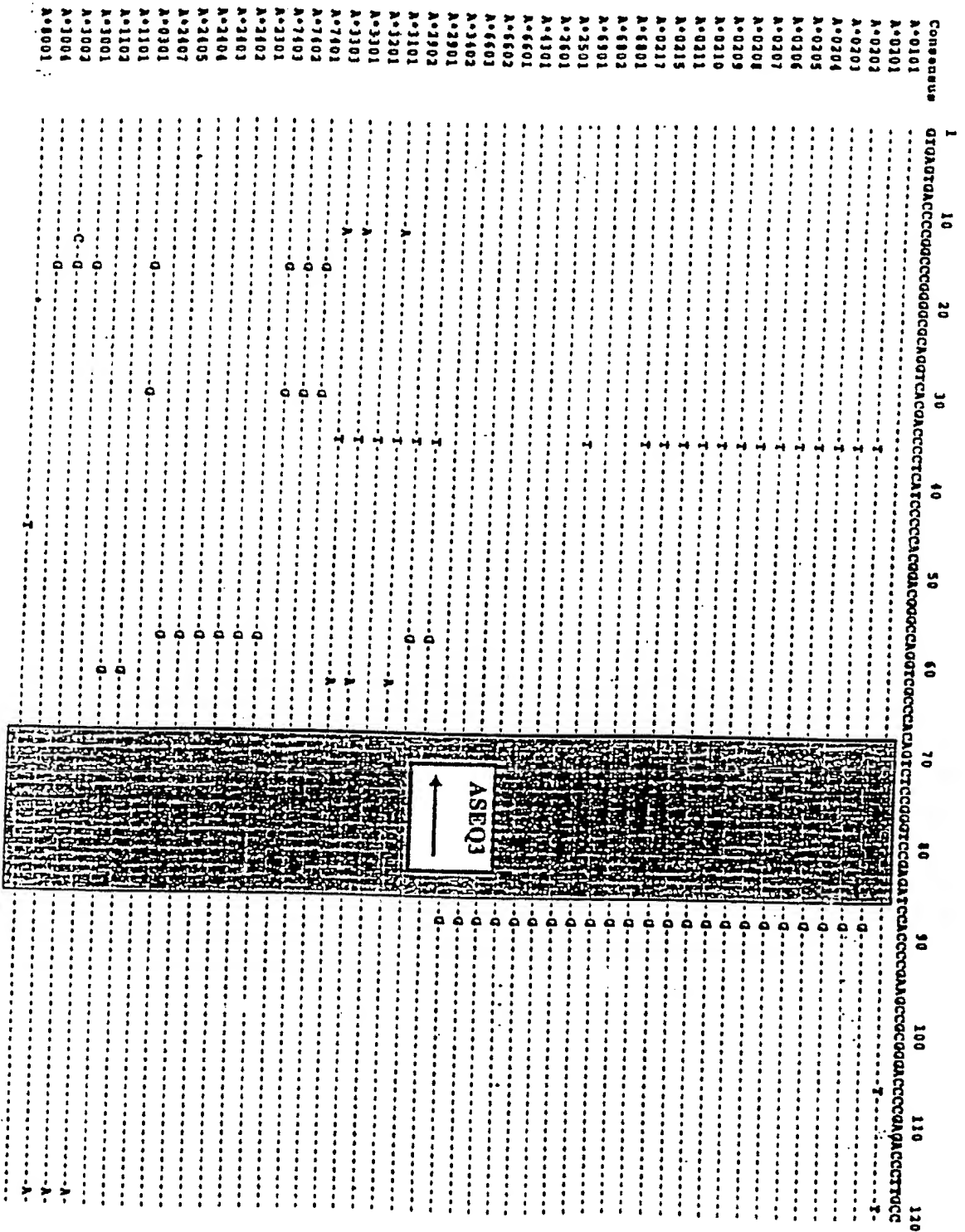


Abbildung 15

Abbildung 15 (Fortsetzung)

44-38861-1A

[illegible]

HLA-A Intron 3, Teil II

Figure 6c2

	130	140	150	160	170	180	190	200	210	220	230	240	250
Consensus	TCCTCTGAGATTCCCAATCTCTGTACCGAGAGAGTGAAGTCCGCTCTCTGTGAGCAATTAGAGGATTAATATCTGAAAGATGACGGAGAACGATCCCTGGAATGCTGATGAT												
A*0101
A*0301
A*1101
A*1102
A*1601
A*3002
A*3004
A*0201
A*0202
A*0203
A*0204
A*0205
A*0206
A*0207
A*0208
A*0209
A*0210
A*0211
A*0212
A*0213
A*0217
A*6801
A*6802
A*6901
A*3101
A*3102
A*3103
A*3104
A*3105
A*3107
A*3501
A*3601
A*3102
A*4101
A*6601
A*6602
A*6603
A*2301
A*2302
A*3101
A*3201
A*3301
A*3303
A*7101
A*7102
A*7103
A*8001

Abbildung 23 (Fortsetzung)

[illegible]

Abbildung 23 (Fortsetzung)

1965-1971

Phylogenetic tree of the first 450 bp of the HLA-A 5' flanking region

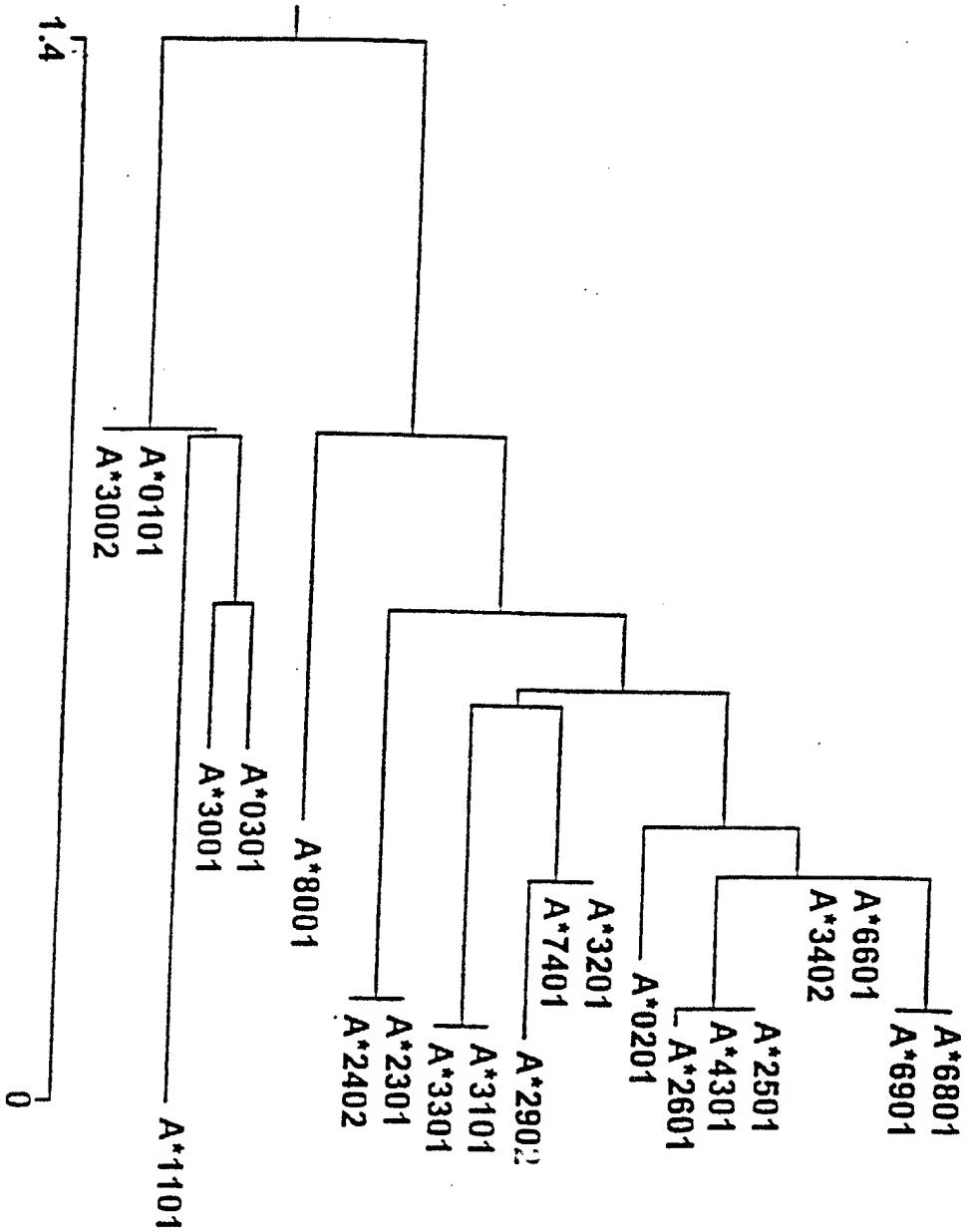


Figure 7

Phylogenetic tree of introns 1-3 of the HLA-A gene

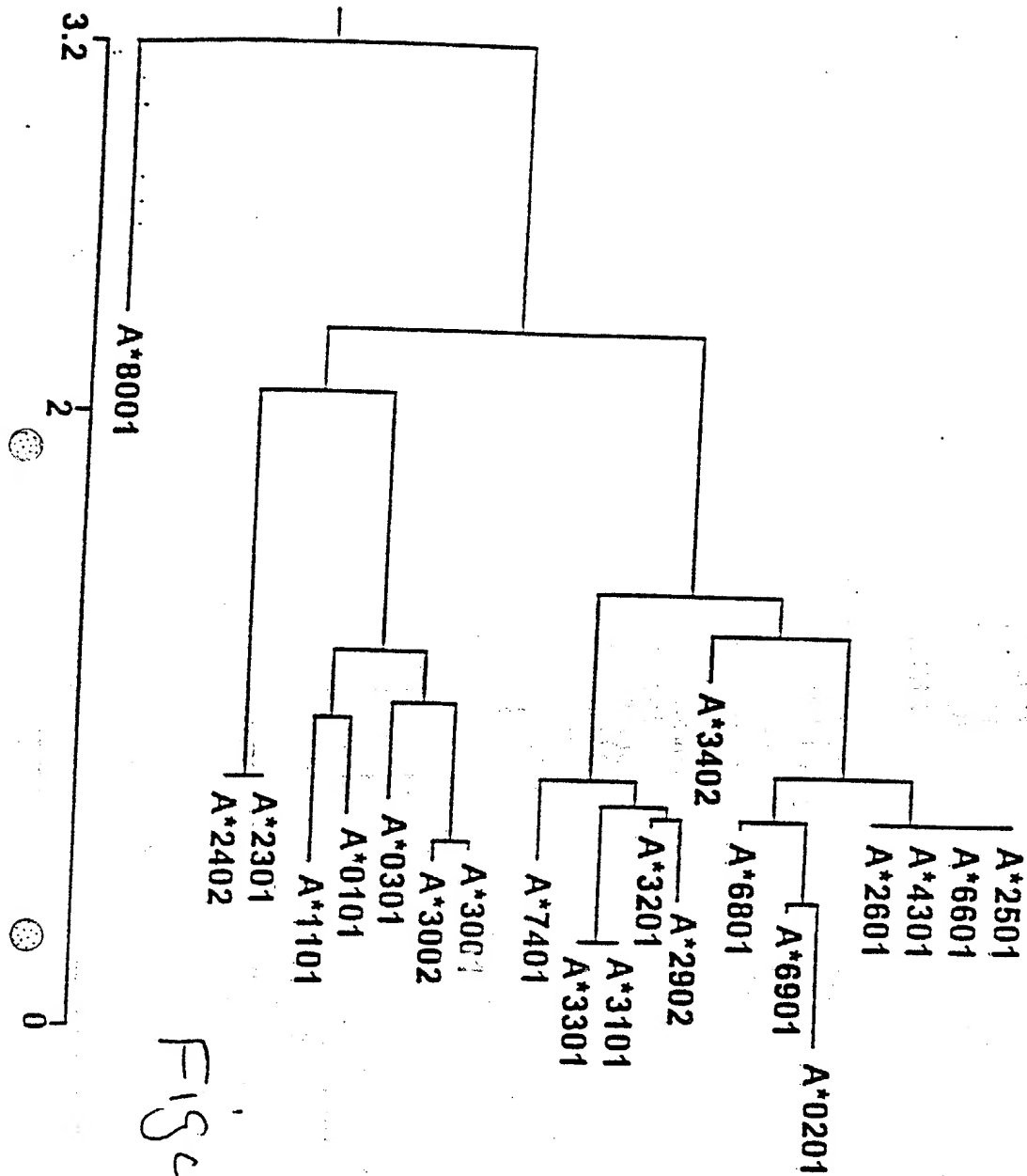
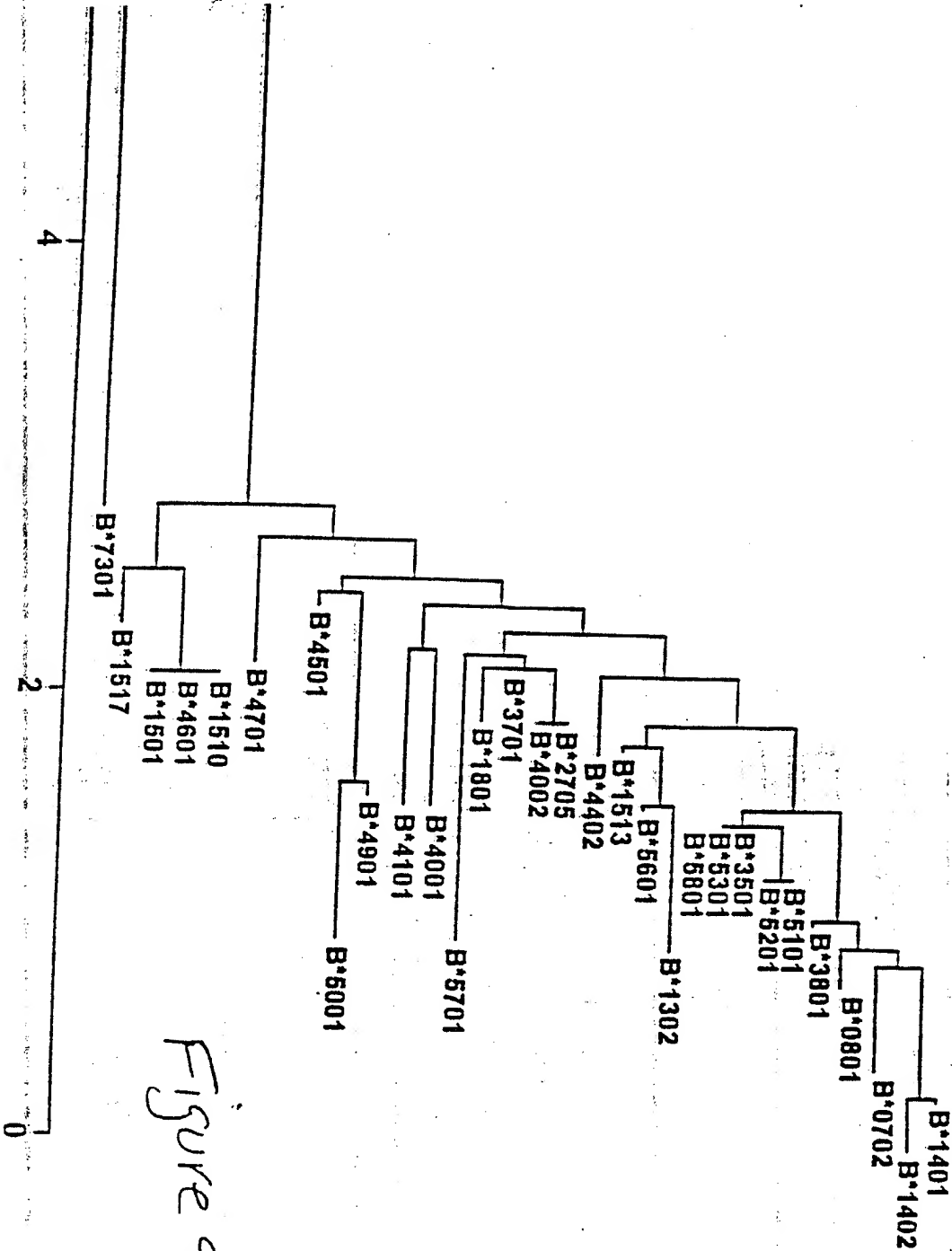


Figure 8

Phylogenetic tree of introns 1-3 of the HLA-B gene



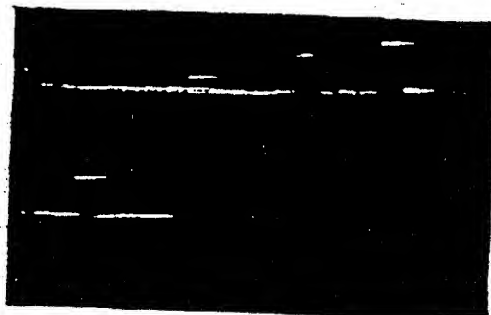


Figure 10.

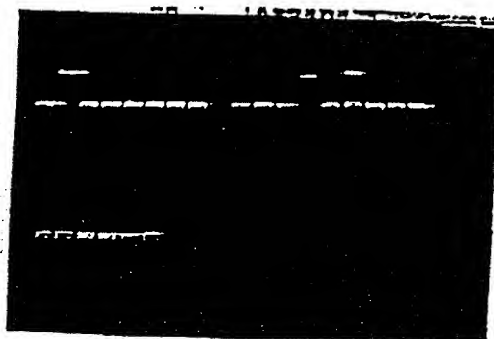


Figure 11.

11-A-B Section 2, part 1

75025 13(A)

CONFIDENTIAL

[illegible]

III.A-B Intron 2, part II

Figure 13(b)

SECRET

Consensus
P0702
00001
00101
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HLA-B Intron 3, part II

FIGURE 14(b)

SECRET

Address	Hex Data	Disassembly
00000000	00000000	00000000
00000001	00000000	00000000
00000002	00000000	00000000
00000003	00000000	00000000
00000004	00000000	00000000
00000005	00000000	00000000
00000006	00000000	00000000
00000007	00000000	00000000
00000008	00000000	00000000
00000009	00000000	00000000
0000000A	00000000	00000000
0000000B	00000000	00000000
0000000C	00000000	00000000
0000000D	00000000	00000000
0000000E	00000000	00000000
0000000F	00000000	00000000
00000010	00000000	00000000
00000011	00000000	00000000
00000012	00000000	00000000
00000013	00000000	00000000
00000014	00000000	00000000
00000015	00000000	00000000
00000016	00000000	00000000
00000017	00000000	00000000
00000018	00000000	00000000
00000019	00000000	00000000
0000001A	00000000	00000000
0000001B	00000000	00000000
0000001C	00000000	00000000
0000001D	00000000	00000000
0000001E	00000000	00000000
0000001F	00000000	00000000
00000020	00000000	00000000
00000021	00000000	00000000
00000022	00000000	00000000
00000023	00000000	00000000
00000024	00000000	00000000
00000025	00000000	00000000
00000026	00000000	00000000
00000027	00000000	00000000
00000028	00000000	00000000
00000029	00000000	00000000
0000002A	00000000	00000000
0000002B	00000000	00000000
0000002C	00000000	00000000
0000002D	00000000	00000000
0000002E	00000000	00000000
0000002F	00000000	00000000
00000030	00000000	00000000
00000031	00000000	00000000
00000032	00000000	00000000
00000033	00000000	00000000
00000034	00000000	00000000
00000035	00000000	00000000
00000036	00000000	00000000
00000037	00000000	00000000
00000038	00000000	00000000
00000039	00000000	00000000
0000003A	00000000	00000000
0000003B	00000000	00000000
0000003C	00000000	00000000
0000003D	00000000	00000000
0000003E	00000000	00000000
0000003F	00000000	00000000
00000040	00000000	00000000
00000041	00000000	00000000
00000042	00000000	00000000
00000043	00000000	00000000
00000044	00000000	00000000
00000045	00000000	00000000
00000046	00000000	00000000
00000047	00000000	00000000
00000048	00000000	00000000
00000049	00000000	00000000
0000004A	00000000	00000000
0000004B	00000000	00000000
0000004C	00000000	00000000
0000004D	00000000	00000000
0000004E	00000000	00000000
0000004F	00000000	00000000
00000050	00000000	00000000
00000051	00000000	00000000
00000052	00000000	

HLA-B intron 3, part III

Figure 14 (c)

三、

[illegible]

THE

Figure 14D

28 / 29

FIGURE 14E

HLA-B Intron 3, part V

CONFIDENTIAL

	510	520	530	540	550	560	570	580
Consensus	GTGACATGGGTGCTGCTTAAAGGTGGTCCCTATGATATGCTCAAGAGCCGCTGATATTTCTATGCTCTCCATGCA							
B*0702
B*0801
B*1302
B*1401
B*1402
B*1501
B*1502
B*1510
B*1512
B*1517
B*1525
B*1601
B*27052
B*27053
B*3501
B*3502
B*3503
B*3701
B*3801
B*3803
B*3906
B*4001
B*4002
B*4101
B*4102
B*4201
B*4402
B*4601
B*4603
B*4604
B*4701
B*4801
B*4901
B*5101
B*5108
B*5201
B*5301
B*5501
B*5601

INTERNATIONAL SEARCH REPORT

In national Application No
PCT/CA 98/00768

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KLOTSCH K ET AL: "Sequencing of HLA Class I genes based on the conserved diversity of the noncoding regions: Sequencing based typing of HLA-A gene" TISSUE ANTIGENS, vol. 50, no. 2, August 1997, pages 178-91, XP002070449 whole article, especially line 1 of the "Abstract"	1-7
Y	CEREB N ET AL: "NUCLEOTIDE SEQUENCES OF MHC CLASS I INTRONS 1,2, AND 3 IN HUMANS AND INTRON 2 IN NONHUMAN PRIMATES" TISSUE ANTIGENS, vol. 47, no. 6, June 1996, pages 498-511, ERRATUM 235/236, XP002070446 see the whole document --- -/-	1-7

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- "Z" document member of the same patent family

Date of the actual completion of the international search

23 December 1998

Date of mailing of the international search report

12/01/1999

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Osborne, H

INTERNATIONAL SEARCH REPORT

In. ational Application No

PCT/CA 98/00768

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	CEREB N ET AL: "DIMORPHIC PRIMERS DERIVED FROM INTRON 1 FOR USE IN THE MOLECULAR TYPING OF HLA-B ALLELES" TISSUE ANTIGENS, vol. 50, no. 1, July 1997, pages 74-76, XP002070448 see the whole document	1-7
X	WO 97 23645 A (SLOAN KETTERING INST CANCER) 3 July 1997 see whole document, especially Seq ID no. 25 and 27 and claim 13: Seq Id No 25 and 27 shows 100% identity in 18bp and 19bp overlap respectively to II-B168 and I3-b126 (i.e. claims 8-14)	8-14
Y	WO 97 20070 A (ANTHONY NOLAN BONE MARROW TRUS ;ARGUELLO RAFAEL (GB); MADRIGAL ALE) 5 June 1997 intron 3 primer of example 1, page 35 shows 100% identity in 19bp overlap to I3-B126	1-7

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INTERNATIONAL SEARCH REPORT

Information on patent family members

In tional Application No
PCT/CA 98/00768

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 9720070 A	05-06-1997	AU 7703896 A EP 0876508 A	19-06-1997 11-11-1998

Form PCT/ISA/210 (patent family annex) (July 1992)